A TOXICITY STUDY ON

PUTTRU PATHANGAM

(DISSERTATION SUBJECT)

For the partial fulfillment of requirements to the degree of

DOCTOR OF MEDICINE (SIDDHA)

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CONTENTS

INTRODUCTION .I

Siddha system of medicine is part and parcel of the earliest civilization of the southern peninsula of India. The Siddha medical system has been practiced from time immemorial by using substances of all possible origins in a way that balances the possible harmful effect of each substance. This form of medicine was professed and practiced by Siddhars who were aware of the constituents of the body and the mysteries of the mind. Their thoughts and teachings were crystallized in the form of a great system of medicine. According to Thirumoolar,

மறுப்பது உடல்நோய் மருந்தென லாகும்
மறுப்பது உளநோய் மருந்தெனச் சாலும்
மறுப்பது இனிநோய் வாரா திருக்க
மறுப்பது சாவை மருந்தென லாமே.

(Thirumanthiram)

Siddha system of medicine is not purely a system of medicine; it is a way of life. If one goes through the available literatures of ancient Tamil Medicine, one would see that it covers all aspects of human life right from, conception in the mother's womb to occurrence of death. It emphasis the regularity and habits of life. The aim of the system is to keep the body and mind in good condition. Karpam, Yogam, Pranayamam and Varmam are also performed in Siddha system of medicine. Siddha is a life science which includes in its fold all the principles governing the mechanism of body and mind in health and sickness. Siddhars classified body temperament based on three humoural theory, such as Vatham, Pitham and Kapham respectively. The medical heritages of Tamils are preserved in the Siddha system.

வேர்பாரு தழைபாரு மெல்ல மெல்ல
பற்பச் செந்தூரம் பாரே.

(Theran paadal)

Having known that the medicinal herbs are not enough to cure the certain chronic and dreadful ailments completely, the Siddhars have made a thorough study on the utility of mineral substances and formulated the medical preparations consisting of Metals (ulogham), Inorganic secondary minerals (uparasam), Organic salts (karasaram), Arsenic compounds (Paadanam) and there by attained prominence in the field of medicine.

சூதகந்தி தாதுபற்பஞ் சொன்னநாட் டார்சிகிச்சை
ஒதரிய மூலியிம்மண் ணூர்ச்சிகிச்சை - வேதடரும்
சத்திரசா ராக்கினிநி சாசரச்சி கிச்சையென்ரே
மூத்தரத்த தாகும் மொழி.

-பதார்த்த குண சிந்தாமணி.

Siddha preparations are made by using mercury, uparasam (secondary minerals), sulphur and other metals, salts and toxic substances such as wax form of medicines (mezhugu), medicated oils (chudar thailam), sublimates (pathangam), hardened medical stones (Kattu), calcinated powders (chunnam) etc.

Preparations are made mainly out of the parts of the plants and trees, mineral and also some animal substances. The use of metals like gold, silver and iron powders in some preparations is a special feature of Siddha medicine.

Every year, hundreds and thousands of people develop cancer. In the year 2000, approximately 10 million new cases of cancer were diagnosed, and there were 6 million cancer-related deaths. Taken together, 22 million people were living with cancer that had been diagnosed within the previous 5 years. These figures reflect a 22% increase in cancer incidence and mortality in the world in comparison with the year 1990.

Cancer is projected to become the leading cause of death worldwide in the year 2010, according to a new edition of the ***World Cancer Report*** from the International Agency for Research on Cancer. Based on projections, cancer deaths will continue to rise with an estimated 9 million people dying from cancer in 2015, and 11.4 million dying in 2030.

On a global basis, Cancer is on the rise, and is assuming alarming, dimensions. Progressive and disseminated malignant disease has a substantial impact on a cancer patient's quality of life, and many cancer treatments may have severe side effects. The rapid increase in the global cancer burden represents a real challenge for health systems worldwide. Our ancestors had left us so many hand-outs to treat various types of cancer by the way of palm manuscripts. In Siddha system, cancer is mentioned as - Puttru, Vippuruthi, Dhurmaangisam, Vansilanthi and so on. The medicine Puttru Pathangam mentioned in the Siddha text to treat Puttru is used since quite a lot of years.

The consequently, education, training and research in this area have not been accorded due attention and support. There is a proverb in Tamil that even the Ambrosia will become poison if it taken in excess. "அளவுக்கு மிஞ்சினால் அமிர்தமும் நஞ்சாகும்." However, in the early 1500s, it was apparent to the physician, Paracelsus, that "the dose differentiates a poison and a remedy" stated more directly, any chemical can be toxic if the dose is high enough.

According to Siddha literature Puttru pathangam is efficient in management of cancer and its related complications. In this aspect Puttru pathangam may be a better choice of drug. Since modern society is against the usage of metallic and herbo-mineral compounds as medicine these study is more important for its own. The quantity and quality of the safety and efficacy data on traditional medicine Puttru pathangam are far from sufficient to meet the criteria needed to support its use.

Here, we take the opportunity to reveal the important of this medicinal preparation and its toxicity evaluation by animal experiment. This anchoring in identity, added to the renewal of interest in such traditional medicines, contributes to the revitalization of Siddha to all life worldwide.

It is time to truly begin our work on bringing our Siddha Traditional medicine - for Puttru and create an equitable system of health to the Society. The future possibilities for the integration of traditional medicine place us at an exciting juncture.

This claim is especially relevant in the case of mercury which is relatively often used in the system; medicine containing purified mercury should only be received, if at all, from a highly qualified practitioner of the art.

AIM AND OBJECTIVES. II

AIM

The aim of this study is to evaluate the safety profile of Puttru Pathangam.

OBJECTIVES

Based on the literature evidences, various sources of ingredients have been collected and purified. After that, the preferred of ingredients will be chosen and used in the preparation of Puttru Pathangam as per literature. Then, the finished product of Puttru Pathangam is sent for elemental analysis to estimate the presence of any heavy metals and other organic and inorganic compounds.

To analysis its safety, Puttru Pathangam is subjected for Acute oral toxicity and Repeated oral toxicity evaluation under OECD guideline on rodents.

Puttru Pathangam has been evaluated in the following aspects:

- Collection of relevant literatures regarding the ingredients of the study drug.
- Getting authentication of raw drugs.
- Purification process of ingredients of Puttru Pathangam.
- Preparation of Puttru Pathangam as per textual method.
- Evaluation of physico-chemical analysis in Puttru Pathangam.
- Elemental analysis of Puttru Pathangam
- Safety study of Puttru Pathangam on rodents.

3.1 RASAM

(Hydrargyrum, Mercury)

Introduction:

Rasam, Hydrargyrum(Hg) - one of Pancha Sootham, is obtained in large quantities from China and Japan both in its native state and combined with sulphur as Cinnabar or red sulphide of mercury. Mercury means life. It is supposed to produce gold from base metals. It is called the expelles of diseases. It occupies central place in Siddha medicine and it could make the body resistant and immortal. It is mentioned as Karpam, Sootham, Sathu, Bharatham, Sivam, Saaru etc. The mercury obtained from Lingam (Cinnabar) is considered as pure and suitable for medicinal purposes.

General properties of Rasam:

விழிநோய் கிரந்திகுன்மம் மெய்ச்சூலை புண்குட்
டழிகாலில் விந்துவினால் அத்தை - வழியாய்
புரியுவிதி யாது புரியுனோ யெல்லாம்
இரியுவிதி யாது மில்லை.

(Gunapaadam, Thathu-Jeeva vaguppu)

Proper use of mercury as medicine cures the diseases of eyes, syphilis, eight types of ulcers (Gunmam), throbbing pain (Soolai), chronic ulcer (Perum pun) and Hansens diseases (Thozhu noi).

Purification methods:

No medicine is so often found adulterer as mercury. Purification is very important before the drug preparations. So many methods are explained in the text.

1. Rasam (35gms) is taken in a mud pot and 166.5gm of Thumbai (Leucas aspera) whole plant juice is poured and it is kept in Sunlight for 10 days. Fresh juice of Thumbai is added daily. After completion, the Rasam containing pot is dried without the juice.

2. Rasam is taken in a Bronze vessel and mixed with Manjal (turmeric) powder, and filtered by using a clean cloth.

3. Required quantity of mercury is placed in a thick cloth and squeezed for 1000 times. Then it is placed in a earthen pot. Fresh water is poured in the earthen pot up to the level of 1inch above the mercury level. The pot is heated with low intensity fire. The water level is maintained by adding water. When the water turns into black in colour, the mercury is separated and washed with vinegar for 4 or 5 times to get the purified and detoxified mercury.

Beneficial properties:

It purifies blood.

It improves Blood and Sperms

Stimulates appetite

It cures the diseases of the internal and external organs of the body

It improves facial complexion

It improves memory power, eradicates amnesia

It strengthens the nerve plexuses.

It develops wisdom through concentration of mind

It prevents senility and increases the life span.

Harmful properties of impure mercurial preparation:

If the medicinal mercury is not purified properly, the disease like bleeding, dropsy, anaemia, excessive body heat, sweating, diarrhoea, thirst, flatulence, blabbering, skin diseases burning sensation of the limbs head diseases, fever, shivering, hiccough etc., will manifest and finally death will occur. The whole body will be burnt and all the teeth will fall down.

Antidotes for Mercury:

If the mercurial poison affects the gluteal region, Sayapattai *(dye plant root bark)* is powdered and given along with jaggery.

For Fixation of teeth and jaws, the stem juice of Kovai thandu *(Coccina indica)* may be poured on the tongue.

If there is burning sensation in limbs, urticaria, dryness of the throat and the patient is unconscious (amnesia), Arugan kizhangu (*Cynodon dactylon*) is triturated and dissolved in goat's milk or cow's milk or butter milk, or cotton seed milk is filtered and administered.

சேரிரதங் கொண்டோர்க்குச் செப்புவாய் வெண்சங்கின்
சாறாகுங் காரவல்லிச் சாறாகும் - சீர்நீலி
நல்வேரை வெந்நீரில் நற்சுண்டை காயளவு
முந்நா ளருந்தல் முறை.

(Agathiyar vida pirathi vida thirattu)

The leaf juice of Vellai Mutsangu *(Azima tetracantha)* or MithiPaaghal *(Momordica muricata)* 80ml oraly twice a day for 3 days.

Avuri (*Indigofera tinctoria)* root bark is triturated with hot water and made into poultice. It is given in the size of Sundaikaai twice a day for three days.

Some preparations:

1. Veera mezhugu

Dosage : 65-130mg

Anupaanam : palm jiggery.

Therapeutic uses : Nachu Paandu, Mahodharam.

*Reference: Anoboga vaithiya navaneetham.

2. Akkini kumara mathirai

Dosage : 65mg.

Therapeutic uses : Paandu, Sobai, Kaamalai.

*Reference: Kannusamy parambarai vaithiyam, pg no: 234.

3. Dasamuga udaya akkini chenduram

Dosage : 488mg.

Anupaanam : Ghee, Honey.

Therapeutic uses : Paandu, Sobai, Kaamalai.

*Reference: Sikicha rathna deepam, pg no: 264.

4. Soodha parpam

Dosage : ½ - Kundri.

Anupaanam : Ghee, Honey.

Therapeutic uses : Mega viranangal, Kiranthi, Korukku.

*Reference: Sikicha rathna deepam, part 2-Vaithiya chinthamani, pg no: 216.

5. Pancha Paadana chenduram

Dosage : Arisi edai.

Duration : 5-7 days.

Therapeutic uses : Puttru, Kuttam, Sanni, Solai.

*Reference: Sikicha rathna deepam,part 2- Vaithiya chinthamani, pg no:229.

MERCURY

Introduction:

Mercury is a metallic chemical element identified by the symbol Hg on the periodic table. It is silver in colour and, unlike other metals, is liquid at room temperature. The ancient name for mercury was quicksilver, meaning "living" silver. This name reflected mercury's lustrous silver colour and its unusually lively behavior: when it is

poured onto a smooth surface, it forms beads that roll rapidly around. The element's modern name comes from Mercury (Mercurius), the fleet-footed messenger of the gods in Roman mythology.

Scientific classification:

Name	: Mercury
Symbol	: Hg
Atomic Number	: 80
Atomic Mass	: 200.59 amu
Melting Point	: -38.87 °C (234.28 K, -37.966 °F)
Boiling Point	: 356.58 °C (629.73 K, 673.844 °F)
No. of Protons/Electrons	: 80
No. of Neutrons	: 121
Electron configuration	: [Xe] 4f14 5d10 6s2
Classification	: Transition Metal
Crystal Structure	: Rhombohedral
Density at 293 K	: 13.456 g/cm3
Colour	: Silver

Source:

- Mercury's presence in the Earth's crust is relatively low compared to other elements. However, mercury is not considered rare because it is found in large, highly concentrated deposits.
- Nearly all mercury exists in the form of a red ore called cinnabar, which is composed of mercury and sulphur.
- Sometimes shiny globules of mercury appear among outcrops of cinnabar, which is probably why mercury was discovered so long ago. The metal is relatively easy to extract from the ore by applying heat and a filtration process.

- First the ore is heated in an oxygen furnace. The mercury is released as fumes and those fumes condense into soot on a water-cooled metal condenser. The mercury is then removed from the soot by a filter system and purified in a vacuum.
- Much of the world's mercury has traditionally been mined in Spain and Italy, though several other countries also produce commercial quantities.

Uses of Mercury:

- Mercury is used in barometers and manometers (instruments for measuring the pressure of gases and liquids), because of its high density.
- The metal also has a high rate of nearly linear thermal expansion, so it is used extensively in thermometers.
- Its ease in amalgamating with metals is made use of in extracting gold, silver, and platinum from their ores.
- Mercury is widely used in making advertising signs, mercury switches, mercury-vapour lamps and other electrical apparatus.
- Various compounds of mercury are used in medicine, dentistry, cosmetics (mascara) and also in agriculture to make fungicides.

TOXICOLOGICAL ASPECTS:

Mercury is a metallic element that occurs naturally in the environment. There are three primary categories of mercury and its compounds:

- Elemental mercury, which may occur in both liquid and gaseous states;
- Inorganic mercury compounds, including mercurous chloride, mercuric chloride, mercuric acetate, and mercuric sulfide; and organic mercury compounds.

Action:

The mercuric ion binds with the sulphydryl groups of enzymes and cellular proteins, mitotic apparatus interfering with enzyme and cellular transport functions. It is rapidly converted to mercuric ions in the blood which can lead to renal tubular damage.

Toxicokinetics of elemental mercury by the human body

Elemental form of Hg	Ways of toxicokinetics by the human Organism	References
Absorption and adsorption	The respiratory absorption of elemental mercury is the major way of absorption in humans (>75-85% of the total uptake). Through the lungs it is eventually transported to the bloodstream. Gastro intestinal tract absorption is negligible (0.01%).	Loredo et al., 2003; DeRouen et al., 2002; Finkelman et al., 2002.
Excretion	Elemental Hg is excreted from the human body in the urine, feces, expired air, sweat and saliva. Variations of the form of excretion depend on the degree of oxidation of elemental Hg to mercuric mercury. In general, a low level exposure is related to a, mainly, fecal excretion, while a high-level exposure is related to a mainly urinary.	Roels et al., 1991.
Distribution	It is mainly transported by red blood cells (>98% of the total uptake) and accumulates mainly on the cerebral gray matter (especially the fetal during pregnancy).	US EPA, 1997c; Hursh et al., 1988.
Biological action	It easily enters tissue and red blood cells where is oxidized to bivalent mercury, with the help of a catalase.	Halbach and Clarkson, 1978.
Affected organs	In a brief, high-level exposure, the lungs. In a long-term exposure the Central Nervous System (CNS) and especially the brain.	Karimi et al., 2002; Adams et al., 1983.

Detoxification and suppression	Vitamin E is reported to be a protective agent. Additionally, ethanol reduces the human organism ability for absorption of elemental mercury, possibly by suppressing the activity of the catalase, which oxidizes it to produce bivalent Hg. Tellurium also appears to have a protective role.	Dunn et al., 1981.
Borderline concentrations	No symptoms reported on <10 μg Hg m-3 of air. Symptoms of micro-mercurism on 10- 50 μg Hg m-3 of air. Symptoms of mercurism on 60-100 μg Hg m-3 of air. Symptoms of the CNS 100-270 μg Hg m-3 of air. A concentration of 10-70 μg g-1 (w/w) in the kidneys causes proteinuria. Concentrations of 70-140 μg l-1 in the bloodstream or 300-600 μg l-1 in the urine cause tremor.	US APA, 1997c; Stratis and Zachariades, 1989; Pavlogeorgatos, 2001.

In General:

Elemental mercury is lipid soluble and easily penetrates biological membranes, including the blood–brain barrier. Metabolism of mercury compounds to other forms of mercury can occur within the tissues of the body.

Elemental mercury can be oxidized by the hydrogen peroxide-catalase pathway in the body to its inorganic divalent form. After exposure to elemental mercury or inorganic mercury compounds, the main route of excretion is via the urine. Determination of concentrations in urine and blood has been extensively used in the biological monitoring

of exposure to inorganic forms of mercury; hair mercury levels do not reliably reflect exposure to elemental mercury or inorganic mercury compounds.

Neurological and behavioural disorders in humans have been observed following inhalation of elemental mercury vapour, ingestion or dermal application of inorganic mercury-containing medicinal products, such as teething powders, ointments, and laxatives, and ingestion of contaminated food.

Specific neurotoxic symptoms include tremors, emotional lability, insomnia, memory loss, neuromuscular changes, headaches, polyneuropathy, and performance deficits in tests of cognitive and motor function.

Although improvement in most neurological dysfunctions has been observed upon removal of persons from the source of exposure, some changes may be irreversible.

Acrodynia and photophobia have been reported in children exposed to excessive levels of metallic mercury vapours and/or inorganic mercury compounds. As with many effects, there is great variability in the susceptibility of humans to the neurotoxic effects of mercury.

The primary effect of long-term oral exposure to low amounts of inorganic mercury compounds is renal damage.

Inorganic forms of mercury have also been associated with immunological effects in both humans and susceptible strains of laboratory rodents, and an antibody-mediated nephrotic syndrome has been demonstrated through a variety of exposure scenarios.

At high levels of exposure, elemental mercury induces adverse effects in most organ systems in the body. Respiratory failure, cardiac arrest, and cerebral oedema are the causes of death in fatal cases.

3.2 GANDHAGAM

(Sulphur)

Introduction:

Gandhagam is a Paadanam which exists as a natural production and occurs abundantly. It is also known as Sakthi, Kaarilayin naatham, Naatham, Naatram, etc. It is bitter and astringent in taste. Its actions are laxative, tonic and antiseptic. It increases various secretions of body including skin. It is excreted through sweat, milk and urine.

It is of four different types as Pirappu, Vaippu, Kolithalai vaippu and Vaanaganthi vaippu.

Therapeutic Uses:

நெல்லிக்காய் கந்திக்கு நீள்பதினெண் குட்டமந்தம்
வல்லை கவிசைகுன்ம வாயுகண்ணோய் - பொல்லா
விடக்கடிவன் மேகநோய் வீறுசுரம் பேதி
திட க் கிரக ணீகபம்போந் தேர்.

(Gunapaadam, Thathu-Jeeva vaguppu)

It is considered to useful in the treatment of 18 types of skin diseases, liver enlargement, abdominal distension, eye diseases, chronic venereal diseases, chronic diarrhoeas, gastric ulcer, poisonous bites, fever due to vatham, etc.

Gandhagam is chiefly used in scabies and other skin diseases both as an external and an internal remedy. The fumes of burning sulphur are said to cure gout and rheumatic affections.

When it is mixed with coconut oil, it is applied to itches and sores; with neem oil to rheumatic affections and with gingili oil and with powdered black cumin seed, it is given internally for venereal herpes.

Gandhaga Parpam is prescribed for leprosy, jaundice, contracted limbs, venereal diseases, psychiatric disorders (Pramai) etc.

Purification methods:

Gandhagam is used in medicine only after it is purified and cleaned according to the rules laid down in Tamil medical science.

1. The Karkam of Lawsonia inermis (Maruthonri) is mixed in cow's curd and placed in a mud pot. The mouth of the pot is covered with a cloth. Sulphur is placed over the cloth. The pot is covered with another pot and burried in the ground. The outer pot is subjected to Pudam with five cow-dung cakes. The Sulphur which melts and settles down is collected. This procedure is repeated for seven times.

2. Sulphur is placed in an iron spoon. A small quantity of cow's butter is added and the spoon is heated till the butter melts; this mixture is immersed in inclined position in cow's milk. This procedure is repeated for 30 times to get purified sulphur. For each time of the process, fresh milk is to be used.

3. Sulphur is melted in the stem juice of Plantain (Vaazhai) tree for ten times to get it purified.

Toxic symptoms of Gandhagam:

Sulphur is not a highly toxic substance. Improperly purified and irregularly prepared Sulphur medicine if consumed over a long period then it causes toxic effects.

Yellowish discolouration of conjunctiva, Pallor of the face, Discolouration of skin like ridged guard flower (Peerkku), Disfigured blackish teeth, Urine like goat's urine, Smoke like odour in the mouth. Feeling of hungry, stomach pain and distension, Eruption on the body are some of the toxic symptoms.

Antidotes for Gandhagam:

செய்யகந்தி தின்றோர்க்குச் செப்புவா யாவிரம்வேர்
தைவேளை வேர்நீலி தன்மூலம் - துய்யசுக்கு
நல்ல பருத்தியிலை நாகேசுரம் சமனா
மல்ல குடிநீரிட்டு வார்.

வார்ப்பா யிளநீரில் வண்டா மரைவிதையை
நேர்ப்பா யரைத்துவடி நீருண்பாய் - கார்ப்பான
நன்மிளகு நீலியின்வேர் நற்சீ ரகஞ்சமனாம்
மன்னீரிற் காய்ச்சியதை மாட்டு.

(Agathiyar vida pirathi vida thirattu)

1. Root bark of Cassia auriculata *(Avarai),* Gynandropis pentaphylla *(Thaivaelai),* and Indigofera tinctoria *(Avuri),* Dry ginger *(Chukku),* Cotton leaves and Mesua Ferrea flower *(Sirunagapoo)* are taken in equal parts and the decoction is prepared.
2. Paste of Thaamarai seeds *(Lotus)* in tender coconut water also used as an antidote.
3. Decoction is made by boiling pepper, Indigofera tinctoria *(Avuri)* root bark and Cumin seeds and it is given.
4. 10 grams in each of Solanum nigrum *(Manathakkali)* root bark, Piper longum *(Thippili)* and Glycyrrhiza glabra *(Athimathuram)* are made in to a decoction and given.
5. If cow ghee is continuously taken in, the victim will be relieved from the effect of sulphur poison.

Some preparations containing Ganthagam:

1. Koada sooli mathirai

Dosage	: Kundri.
Anupaanam	: Ghee.
Therapeutic uses	: Atta gunmam, Vayu rogam.

*Reference: Sarabaenthirar vaithiya muraighal – gunma roaga sikichai.

2. Sura mathirai

Dosage	: Pana eadai.
Anupaanam	: Notchi extract.
Therapeutic uses	: Suram

*Reference: Attavanai vaagadam, pg no: 192.

3. Silokya chinthamani

Dosage : Kudriyalavu; 40 days.

Anupaanam : Honey.

Therapeutic uses : Vatha, Pitha and Kapha diseases.

*Reference: Vaithiya sinthamani, pg no: 128.

4. Vaaivu Kudori

Dosage : Kundriyalavu; 3 days.

Anupaanam : Ginger extract.

Therapeutic uses : Kuttam, Peenism, Elaippu, Erumal.

*Reference: Vaithiya sinthamani, pg no: 128.

5. Kaala megha narayana Chenduram

Dosage : 1/4 -1/2 kundri; twice a day.

Anupaanam : Thuthuvalai kirutham for 15 days.

Therapeutic uses : Silethuma rogam 20, Vaatha rogam.

*Reference: Sikicha rathna deepam, pg no: 247.

SULPHUR

Introduction:

Sulphur is a multivalent non-metal, abundant, tasteless and odourless. In its native form sulphur is a yellow crystalline solid. In nature it occurs as the pure element or as sulfide and sulfate minerals. Although sulphur is infamous for its smell, frequently compare to rotten eggs, that odour is actually characteristic of hydrogen sulphide (H_2S).

Sulphur occurs naturally as the pure element (native Sulphur) and as sulfide and sulfate minerals. Elemental Sulphur crystals are commonly sought after by mineral collectors for their distinct, brightly colored polyhedron shapes.

Description:

Symbol	: S
Atomic number	: 16
Atomic mass	: 32.06 g.mol -1
Density	: 2.07 g.cm -3 at 20 °C
Melting point	: 113 °C
Boiling point	: 445 °C
Isotopes	: 5
Electronic shell	: [Ne] 3s23p4
Standard potential	: - 0.51 V

Occurrence:

- On Earth, elemental Sulphur can be found near hot springs and volcanic regions in many parts of the world, especially along the Pacific Ring of Fire; such volcanic deposits are currently mined in Indonesia, Chile, and Japan.
  Historically, Sicily was a large source of Sulphur in the Industrial Revolution.

- Common naturally occurring Sulphur compounds include the sulfide minerals, suchas pyrite (ironsulfide), cinnabar (mercurysulfide), galena (leadsulfide),sphaler ite (zinc sulfide) and stibnite (antimony sulfide); and the sulfates, such as gypsum (calcium sulfate), alunite (potassium aluminium sulfate), andbarite (barium sulfate).

History:

- A natural form of Sulphur known as *shiliuhuang* was known in China since the 6th century BC and found in Hanzhong. By the 3rd century, the Chinese discovered that Sulphur could be extracted from pyrite. (Zhang,)

- Indian alchemists, practitioners of "the science of mercury", wrote extensively about the use of sulphur in alchemical operations with mercury, from the eighth century CE onwards (White, David Gordon). In the rasaśāstra tradition, sulphur is called "the smelly," (Skt. gandhaka).

- Being abundant in native form, Sulphur was known in ancient times, mentioned for its uses in ancient India, ancient Greece, China and Egypt.
- Fumes from burning Sulphur were used as fumigants, and Sulphur-containing medicinal mixtures were used as balms and antiparasitics.
- Sulphur is referenced in the Bible as *brimstone* (burn stone) in English, with this name still used in several nonscientific tomes (Greenwood, N. N.).
- It was needed to make the best quality of black gunpowder. Elemental Sulphur was once extracted from salt domes where it sometimes occurs in nearly pure form, but this method has been obsolete since the late 20th century.

Applications

- The major derivative of sulphur is sulphuric acid (H2SO4), one of the most important elements used as an industrial raw material.
- Sulphur is also used in batteries, detergents, fungicides, manufacture of fertilizers, gun power, matches and fireworks.
- Elemental Sulphur is mainly used as a precursor to other chemicals. Approximately 85% (1989) is converted to Sulphuric acid -H_2SO_4.

Some Sulphur-containing organic compounds: (Cremlyn R. J)

- Thiols or mercaptans (as they are mercury capturers as chelators) are the Sulphur analogs of alcohols; treatment of thiols with base gives thiolate ions.
- Thioethers are the Sulphur analogs of ethers.
- Sulfonium ions have three groups attached to a cationic Sulphur center. Dimethylsulfoniopropionate (DMSP) is one such compound, important in the marine organ ic Sulphur cycle.
- Sulfoxides and sulfones are thioethers with one and two oxygen atoms attached to the Sulphur atom, respectively. The simplest sulfoxide, dimethyl sulfoxide, is a common solvent; a common sulfone is sulfolane.
- Sulfonic acids are used in many detergents.

Protein and organic cofactors

- Sulphur is an essential component of all living cells. It is the seventh or eighth most abundant element in the human body by weight. A 70 kg human body contains about 140 grams of Sulphur.
- In plants and animals, the amino acids cysteine and methionine contain most of the Sulphur. The element is thus present in all polypeptides, proteins, and enzymes that contain these amino acids. In humans, methionine is an essential amino acid that must be ingested. However, save for the vitamins biotin and thiamine, cysteine and all Sulphur-containing compounds in the human body can be synthesized from methionine.
- Disulfide bonds (S-S bonds) formed between cysteine residues in peptide chains are very important in protein assembly and structure. These covalent bonds between peptide chains confer extra toughness and rigidity. (Nelson, D. L.)
- Eggs are high in Sulphur because large amounts of the element are necessary for feather formation, and the characteristic odor of rotting eggs is due to hydrogen sulfide. The high disulfide bond content of hair and feathers contributes to their indigestibility and to their characteristic disagreeable odor when burned.
- Two of the 13 classical vitamins, biotin and thiamine contain Sulphur, with the latter being named for its Sulphur content. Sulphur plays an important part, as a carrier of reducing hydrogen and its electrons, for cellular repair of oxidation.

Health effects of sulphur:

All living things need sulphur. It is especially important for humans because it is part of the amino acid methionine, which is an absolute dietary requirement for us. The amino acid cysteine also contains sulphur. The average person takes in around 900 mg of sulphur per day, mainly in the form of protein.

Elemental sulphur is not toxic, but many simple sulphur derivates are, such as sulphur dioxide (SO_2) and hydrogen sulfide.

Sulphur can be found commonly in nature as sulphides. During several processes Sulphur bonds are added to the environment that are damaging to animals, as well as

humans. These damaging sulphur bonds are also shaped in nature during various reactions, mostly when substances that are not naturally present have already been added. They are unwanted because of their unpleasant smells and are often highly toxic.

TOXICOLOGICAL EFFECTS

Acute toxicity:

Sulphur is known to be of low toxicity, and poses very little if any risk to human and animal health (The Agrochemicals Handbook.). Short-term studies show that Sulphur is of very low acute oral toxicity and does not irritate the skin. Sulphur also is not a skin sensitizer. However, it can cause some eye irritation, dermal toxicity and inhalation hazards.

When taken orally, it has a mild laxative action. It may cause irritation of skin and the mucous membranes. Sulphur is considered a skin and eye irritant (Worthing, C. R.). Acute exposure inhalation of large amounts of the dust may cause catarrhal inflammation of the nasal mucosa which may lead to hyperplasia with abundant nasal secretions. Trachiobronchitis is a frequent occurrence, with dyspnea, persistent cough and expectoration which may sometimes be streaked with blood.

Chronic toxicity:

Chronic exposure to elemental Sulphur at low levels is generally recognized as safe. Epidemiological studies show that mine workers exposed to Sulphur dioxide throughout their lives often had eye and respiratory disturbances, chronic bronchitis and chronic sinus effects. However, no known risks of oncogenic, teratogenic, or reproductive effects are associated with the use of Sulphur. Also, Sulphur has been shown to be non-mutagenic in microorganisms.

Repeated or prolonged exposure to dust may cause irritation to the mucous membranes. Broncho pulmonary disease may occur which, after several years, may be complicated by emphysema and bronchiectasis. Early symptoms in Sulphur miners often include upper respiratory tract catarrh, with cough and expectoration which is mucoid and may even contain granules of Sulphur. Asthma is a frequent complication. The maxillary

and frontal sinuses may be affected; involvement is usually bilateral and pansinuitis may occur.

Organ Toxicity

Pulmonary function may be reduced. Radiological examinations have revealed irregular opacities in the lungs and occasionally nodulation has been reported, but not true nodular fibrosis.

Effects of sulphur on the environment:

Sulphur can be found in the air in many different forms. It can cause irritations of the eyes and the throat with animals, when the uptake takes place through inhalation of Sulphur in the gaseous phase. Sulphur is applied in industries widely and emitted to air, due to the limited possibilities of destruction of the Sulphur bonds that are applied.

Mothers can even carry Sulphur poisoning over to their children through mother milk. The damaging effects of Sulphur with animals are mostly brain damage, through malfunctioning of the hypothalamus, and damage to the nervous system.

Laboratory tests with test animals have indicated that Sulphur can cause serious vascular damage in veins of the brains, the heart and the kidneys. These tests have also indicated that certain forms of Sulphur can cause foetal damage &congenital effects.

3.3 LINGAM

(Cinnabar, Virmilion)

Introduction:

The mineral substance is red, heavy, brilliant and it is found in native state in quick silver mines. Formerly it was mostly imported from China and Batavia. Lingam is also known as Inkuligam, Raasam, Karpam, Kadai vanni, Maniraagam etc.

Vaipu methods of Lingam are detailed in the texts

Boga munivar sarakku vaippu - 800

Matchamuni – 800, etc.,

General properties of Lingam:

Lingam is effective in the treatment of diarrhoea, pyrexia, delirium, utricaria, dieresis, tuberculosis, unknown insect bites, leprosy, skin diseases.

பேதிசுரஞ் சந்தி பெருவிரண நீரொடுத
காதகடி காசங் கரப்பான்புண் - ணோத
வுருவுலிங்க சங்கதமா யூறுகட்டி யும்போங்
குருவுலிங்க சங்கதமத்தைக் கொள்.

(Gunapaadam,Thathu-Jeeva vaguppu)

It has the properties of curing the diseases caused by the earth element and cures the diseases caused by the water element.

Purification methods:

- The crude of Lingam is soaked for one day in mother's milk and lemon juice respectively and it is taken.
- Lingam is boiled with Lime stone water, Poosani neer (Benincosa hispida), Cow's milk and Lemon juice and is taken as a purified form.
- Lingam is soaked in Lemon juice and kept in Sunlight (Sooriya pudam).

Toxic symptoms of Lingam:

Loss of taste, difficulty in eating and drinking water, ulcers in the buccal cavity uvula (base of the mouth), inner portion of the tongue, larynx and large intestine, foul odour of the mouth, discharge of viscous-whitish saliva, difficult to speak and burning sensation are the toxic features of it.

Antidotes for Lingam:

Saathikkai (Myristica fragrans), Vaal milagu (Piper cubeba), Root bark of red cotton tree (Gossypium arboreum) and Sugar candy each 4.2 gm are made into a decoction and administered twice a day for 48 days.

Some preparations containing Lingam:

1. Bala sanjeevi mathirai

Dosage : 1 tablet.
Anupaanam : Notchi juice, Tulasi juice, Surasm of Ginger.
Therapeutic uses : 8 types of Maantham
*Reference: Siddha vaithiya thirattu, pg no: 7

2. Linga boopathi mathirai

Dosage : Payaralavu.
Anupaanam : suitable anupaanam.
*Reference: Siddha vaithiya thirattu, pg no: 30.

3. Shanmuga thala chenduram

Dosage : 1/4 - 1/2 Kundri.
Anupaanam : Honey, Ghee.
Therapeutic uses : Chronic non-healed ulcers, Gunmam, Thimir kuttam, Suram, Araiyappu, Kiranthi.
*Reference: Kannusamy parambarai vaithiyam.

4. Sorna Pushpa chenduram

Dosage : 1 Kudri.

Anupaanam : Ghee, Honey.

Therapeutic uses : Kuttam, Karungkuttam, Megaranam, Pouthiram.

*Reference: Kannusamy parambarai vaithiyam.

5. Aanantha vayiravam

Dosage : Milagalvu.

Therapeutic uses : Sanni, Gunmam, Kuttam, Moolam.

*Reference: Agathiyar chenduram 300.

6. Boopathi kuligai

Dosage : Arisi eadai.

Anupaanam : Mother milk.

Therapeutic uses : 13 types of sanni, Thiri thodam

*Reference: Agathiyar vaithiya ratna surukkam.

CINNABAR

Cinnabar is generally found in a massive, granular or earthy form and is bright scarlet to brick-red in color. It occasionally occurs in crystals with a non-metallic adamantine luster.

Cinnabar is the naturally occurring mineral with mercury in combination with sulphur, and is red in color so called red mercury sulphide, Zhu Sha or China Red. Cinnabar ores are the major source for metallic mercury production.

General description:

Category : Sulphide mineral

Formula : mercury (II) sulphide, HgS

Crystal symmetry : Trigonal Trapezohedral

H-M symbol : 3 2

Space group : $P3_1$ 2 1

Color : Cochineal-red, towards brownish red and lead-grey.

Other forms of cinnabar

- Hepatic cinnabar is an impure variety from the mines of Idrija in the Carniola region of Slovenia, in which the cinnabar is mixed with bituminous and earthy matter.
- Metacinnabarite is a black-colored form of HgS, which crystallizes in the cubic form.
- Synthetic cinnabar is produced by treatment of Hg (II) salts with hydrogen sulphide to precipitate black, synthetic metacinnabarite, which is then heated in water. This conversion is promoted by the presence of sodium sulphide.
- Hypercinnabar is crystallised in the hexagonal form.

Historical uses:

Cinnabar is insoluble and stable, and cinnabar powder has been used as an important ingredient in traditional Chinese medicines and in Indian Ayurvedic medicines (Saper RB, Kales SN).

Cinnabar-gold was used as an alchemical drug of longevity, called Makaradhwaja in India (Mahdihassan S.).

Cinnabar is used to colour paints and as one of red colouring agents used in tattoo dyes. Approximately 40 traditional Chinese medicines contain some cinnabar according to Pharmacopeia of China and it is the major source of mercury found in traditional medicines.

Disposition of cinnabar:

The solubility and bioavailability of cinnabar are quite low. The water solubility of mercuric chloride is 30–70 g/L, but cinnabar is less than 0.001 g/L at 20°C.

In the stomach, the lower pH the more cinnabar dissolution as Hg_2SOH^+ formation occurs. In the intestine, sulphur increase cinnabar dissolution as mercury-sulphur complexes such as $Hg\ (SH)^+$, $HgS\ (OH)^-$, $HgS_2\ (OH)^-$, and $HgS_3\ (OH)^-$ are formed (Zeng KW).

Ultrasound can reduce cinnabar particle size and increase cinnabar surface area and isoelectric point.

Absorption

- Absorption of cinnabar (0.2%) from the gastrointestinal tract is much less than mercuric chloride (7–15%), and methyl mercury (>95%). In general, bioavailability of cinnabar is 30- to 60- fold less than mercuric chloride (Schoof RA).
- Nonetheless, both crude cinnabar and synthetic mercury sulphide have very low oral bioavailability and are poorly absorbed from the gastrointestinal tract as compared to mercuric chloride and methyl mercury, but are better than liquid elementary mercury (less than 0.01%). Mercury vapour is readily absorbed (80%) through diffusion in the lungs. When cinnabar is heated, mercury vapour is released, and is easily absorbed to produce local and systemic toxicity.
- This is why in Pharmacopeia of China (Pharmacopoeia of China), heating cinnabar is restricted. Cinnabar is not used in injectable preparations. Little is known about cinnabar absorption via the skin, or from parenteral administration.

Distribution and biotransformation

- The distribution of mercury from absorbed cinnabar basically follows the distribution pattern for inorganic mercurials. The highest concentration of mercury is found in kidney, a major target of inorganic mercury exposure. (Sin YM).
- Inorganic mercury salts do not readily pass blood-brain barrier or placenta. However, a small portion of absorbed inorganic mercury can be reduced in tissues and exhaled as mercury vapour.
- A significant portion of mercury vapour crosses the blood-brain barrier and placenta before it is re-oxidized to divalent inorganic mercury by tissue and erythrocyte catalase. Oral cinnabar or synthetic mercury sulphide administration results in brain distribution (about 10% of renal accumulation), mainly to the cerebral cortex and cerebellum.
- In the Chinese literature, it is assumed that cinnabar could be converted to methyl mercury in the intestine under anaerobic conditions at pH 7 (Liang AH).

However, no evidence is available to support this assumption. Intestinal bacteria can convert methyl mercury to inorganic mercury.

- To better understand toxicokinetics of cinnabar is very important for appropriate safety assessment of mineral cinnabar used in traditional medicines.

TOXICITY PROFILE:

- Cinnabar-containing traditional medicines are generally relatively non-toxic at therapeutic doses. The correct preparation methods, appropriate doses, disease status, age and drug combinations are important factors impacting cinnabar toxicity (Liang AH).
- In general, the adverse effects at therapeutic doses of cinnabar-containing traditional medicines are rare and are largely tolerable and reversible. The cinnabar poisoning cases are associated with overdose, long-term uses, and improper processing such as heating, decocting, fumigating, or in combination with other drugs (Liang AH).
- The long-term use of cinnabar-containing traditional medicines could result in renal dysfunction due to accumulation of mercury in the kidney. Blurred vision due to accumulation of mercury in brain is possible, gastrointestinal symptoms also often occur following long-term administration. Skin allergic reaction may occur when cinnabar is used in tattoo dyes (Bagley MP).
- Inhalation of mercury vapour produces acute corrosive bronchitis and interstitial pneumonitis and, if not fatal, may be associated with central nervous system effects such as tremor or increased excitability. With chronic exposure to mercury vapour, the major effects are on the central nervous system. The triad of tremors, gingivitis and erethism (memory loss, increased excitability, insomnia, depression and shyness) has been recognized historically as the major manifestation of mercury poisoning from inhalation of mercury vapour. Sporadic instances of proteinuria and even nephrotic syndrome may occur in persons with exposure to mercury vapour, particularly with chronic occupational exposure (ATSDR).
- Kidney is the major target organ for inorganic mercury in humans and in experimental animals. Although a high dose of mercuric chloride is directly toxic to renal tubular cells, chronic low-dose exposure to mercuric salts may induce an

immunologic glomerular disease. Exposed persons may develop proteinuria that is reversible after workers are removed from exposure.

- Acrodynia has occurred in children chronically exposed to inorganic mercury compounds in teething powder and diaper disinfectants, as well as to organomercurials. Acordynia is characterized by pink hands and feet (also called Pink Disease). These subjects are photophobic and suffer from joint pains (Clarkson TW).
- The major human health effect from exposure to methyl mercury is neurotoxicity. Clinical manifestations of neurotoxicity include paresthesia (a numbness and tingling sensation around the mouth, lips) and ataxia, manifested as a clumsy, stumbling gait, difficulty in swallowing and articulating words. Other signs include neurasthenia (a generalized sensation of weakness), vision and hearing loss, and spasticity and tremor. There may finally progress to coma and death.

Children are sensitive:

- Early life stages are particularly vulnerable to mercury intoxication.
- Cinnabar-containing Chinese medicines are used in pediatrics, mainly for their sedative and hypnotic effects.
- Toxicity has been reported from inappropriate use of cinnabar and cinnabar-containing medicines in infants and preschool children (Kang-Yum E). Thus, caution should be taken when cinnabar-containing Chinese medicines are used for children, as children are susceptible to mercury toxicity.

Treatment:

- Therapy for mercury poisoning should be directed toward lowering the concentration of mercury at the critical organ or site of injury.
- In acute renal failure, hemodialysis may be the first measure, along with administration of chelating agents for mercury, such as dimercaprol (BAL), 2, 3-dimercaptosuccinic acid (DMSA, succimer), EDTA (calcium disodium, edentate calcium disodium), or D-penicillamine (NAP).
- Succimer (DMSA) is a FDA-approved pediatric use in the treating mercury poisoning.

3.4 THALAGAM

(Yellow arsenic, Arsenic trisulphide)

Introduction:

Thalagam is called in various names such as Arithaaram, Kothandam, Peethagi, Aalambi, Maaldevi, Ponvarni, Maanjal varni. It is as such available alone in India in small quantities. It is also available in combination with iron. Though most of the western countries do not use this medicine for internal administration, it is used for internal administration in small quantities in India. Depending upon the colour, appearance and properties it has been classified into four types.

1. Sivappu Arithaaram - treatment of fever with chills, pricking pain and leprosy.
2. Madal Arithaaram - treatment of asthma, kapham, cough and non healing ulcers.
3. Pon Arithaaram - treatment of high chronic fever, poisonous insect bites.
4. Karattu Thalagam – treatment of cough and eight types of ulcers(gunmam).

General properties of Thaalagam:

தாளகத்தின் பேருரைக்கத் தாலுகவுள் நோய்குட்டம்
நீளக் குளிர்காய்ச்சல் நீடுகபம் - நாளகங்கொள்
துஷ்டப் பறங்கிப்புண் சூழழுகண் மண்டைநோய்
கிட்டப் படுபமா கிளத்து.

(Gunapadam, Thathu –Jeeva vaguppu)

Thaalgam is effective in the treatment of skin diseases, diseases of head and tongue, fever with chills, kapha diseases, veneral(parangi pun) focus ulcer in the urethra etc.

Purification methods:

1. 35 gm of Thalagam is bundled in a cloth and soaked separately in cow's urine, rice water fermentation, lime water, pumpkin juice, cow's milk and decoction of peepul tree(Ficus religiosa) and subjected to Thulayanthiram method. 1.3litres of liquid material is taken in each.

2. 35 gm of Thalagam is burried within lime stone. Palm toddy is poured on the lime stone for 10 times to get purified. Then the Thalagam is washedout and dried.

3. Small pieces of the material is bundled in a double layer cloth and is kept immersed in cow's urine and heated for 3 days. The same process is repeated with the rice cleaned water, vinegar (Kaddi), individually to get purified form.

Toxic symptoms of Thalagam:

An improper purification or administration will cause toxic effect.

The signs and symptoms are burning pain of the stomach, hoarseness of voice, nasal bleeding, bleeding from the nail buds, loss of appetite and loss of smell, indigestion, itching over the body, redness in the tip of the hairs, lower abdominal swelling and throbbing pain in the lumbar regions.

Antidotes for Thalagam:

தேருவாய் தாளகத்தை தின்றோர்க்கு நீலியதன்
வேருடனே யாவிரம்பூ வெட்டிவேர் - சீரகமும்
மாதளைவித் தோடுதென்னம் வங்குரும்பை காசினிவேர்
தீதகல் நற்குடிநீர் செய். ***(Agathiyar vida pirathi vida thirattu)***

கொடிவேலி யாவிரைவேர் கொச்சி மிளகுங்
கடலுப் பிவைகலந்து காய்ச்சி - யுடலினுக்
கூறுசெய்த தாளகத்தா லுற்றவிட நீங்கியிரு
கூறுசெய்து நன்றாய் குடி. ***(Karuvur Devar Thandagam)***

1. Each 10 grams of Indigofera tinctoria *(Avuri)* root bark, cassia auriculata *(Avarai)* flowers, Andropogan muricatus root, *(Vettiver),* Cuminum Cyminum *(Seeragam)* seeds, Punica granatum *(Mathulai)* seeds,tender leaves of Cocos nucifera *(Kurumbai)* - and Cichorium intybus*(Kasini keerai)* root are boiled with water and the decoction is administered. This will neutralize the toxic effects of yellow orpiment.

2. Each 10 grams of root bark of Plumbago zeylanica *(Chithromoolam)* and pepper is boiled in water, made into decoction and 5 grams of Sodium

Chloride is well dissolved and administered daily for 20, 30, or 40 days depending upon the severity of the toxic effects.

Some preparations containing Thalagam:

Thalaga parpam

Dosage : 488mg.
Anupaanam : honey.
Therapeutic uses :Ilaippu, Enburukki, Pramiyangal.
*Reference: Siddha Maruthuva Chinthamani, pg no: 114.

Thalaga karuppu

Dosage : 1-2 ulunthu.
Anupaanam : honey, ghee, thalisathi choornam.
Therapeutic uses :Kaasam, Suvasa kaasam, Suram.
*Reference: Gunapaadam – Thathu jeeva vaguppu, pg no:339.

Thalaga mathirai

Dosage : 1-2, twice a day.
Therapeutic uses : All types of suram.
*Reference: Anuboga vaithiya bramma ragasiyam, pg no: 94.

Arithaara kuligai

Therapeutic uses : Kan kasam used externally with honey.
*Reference: Marunthu seimuraigal, pg no: 104.

YELLOW ORPIMENT

Introduction:

Arsenic trisulphide is a naturally occurring form of trivalent arsenic. Orpiment is one of the major arsenic containing mineral. It is also manufactured from the reaction of arsenic trioxide with sulphur and is available commercially. Arsenic trisulphide is insoluble in water and so poorly absorbed. If therefore represents much less of an acute toxic hazard than soluble arsenic compounds.

Synonyms

Kings gold, Arsenous sulphide, Arsenic sequisulphide, Diarsenic trisulphide, Arsenic sulphide, Arsenious sulphide.

Chemical name and formula

Name : Arsenic tri sulphide

Formula : $As_2 S_3$

Physical properties

Colour	:	Yellow-orange
Molecular weight	:	246.04g/mol
Melting point	:	300ºc
Density	:	$4.25gm/cm^3$
Boiling point	:	707ºc

Origin and History:

It is an historical pigment. It has been identified on ancient Egyptian objects and paintings from the thirty-first to the sixth century B.C. It is mentioned in Greek and Roman literary sources. The Hellenistic Leyden papyrus described its use for late Egyptian painting, as does the Mappae Clavicula for early mediaeval painting. The pigment has been described in various other medieval manuscripts dating from the 12th to the 15th centuries.

Source:

Orpiment is a rare mineral that usually forms with realgar. In fact the two minerals are almost always together. Crystals of orpiment are extremely rare as it usually forms masses and crusts. The masses are sometimes transparent to a degree and have a gemmy quality to them. The yellow color is special to orpiment and can be confused only with a few other minerals. Orpiment is derived from the latin *auripigmentum*, or "golden pigment." The largest quantities of orpiment were found in Turkish Kurdistan (Julamerk), and in the Russian republic Georgia.

The orpiment in Italian painting often came from the fumaroles of mount Vesuvius and from the Campi Flegrei in Tuscany. Since the later Middle Ages the pigment was also artificially made. This pigment would most likely be the result of sublimation of arsenic, or arsenic oxide and orpiment with or without the addition of sulfur. Notable occurrences of orpiment are found today in Kyrgyzstan; Romania; Peru; Japan; Utah, USA; and Australia.

Preparation

Arsenic trisulfide is made when an arsenic compound like arsenic trichloride reacts with hydrogen sulfide. It is also made when arsenic and sulfur are heated together.

Uses

As_2S_3 and As_4S_4 have been investigated as treatments for acute promyelocytic leukemia (APL) (D.-P. Lu, et al). It was looked at for treating cancer and also used in cosmetics.

TOXICOKINETICS

Absorption:

Arsenic trisulphide is poorly absorbed after ingestion .The efficiency of absorption is dependent on particle size; fine powders are better absorbed than larger particles.

Following inhalation irrespirable particles are trapped in the upper airways and deposited in the gastrointestinal tract by mucociliary clearance.

Direct evidence of transcutaneous arsenical absorption in man is scarce.

Distribution:

Absorbed arsenic is distributed to all body tissues. High concentrations would be expected in keratin-rich tissues such as hair, skin and nails due to sulphydryl group binding. Trivalent arsenic is methylated in the liver to methylarsonic acid and a dimethylarsinic acid Short-term study on humans indicate that daily intake in excess of 0.5 mg progressively, but not completely, saturates the capacity to methylate inorganic arsenic.

Excretion:

The half-life of arsenic in blood is about 60 hours with rapid renal excretion predominantly as mono- and dimethyl- derivatives. Small amounts excreated in faeces, suggesting minor biliary clearance.

MECHANISM OF TOXICITY

The principle mechanism of arsenic intoxication is disruption of thiol proteins. For example, arsenic inactivates pyruvate dehydrogenase by complexing with the sulphydryl groups of a lipoic acid moiety (6, 8-dithiooctanoic acid) of the enzyme.

Enhanced cellular destruction of damaged thiol proteins may produce toxic oxygen radicals.

Arsenic-induced reduced lymphocyte proliferation and impaired macrophage function also have been described.

ACUTE TOXICITY

Systemic toxicity may follow arsenic trisulphide ingestion, inhalation or topical exposure.

Topical

Irritant to skin and mucous membranes. Systemic arsenic poisoning may occur after substantial exposure.

Ingestion

Rapid onset (within 1-2 hours) of burning of the mouth and throat, hypersalivation, dysphagia, nausea, vomiting, abdominal pain and diarrhoea. In severe cases gastrointestinal haemorrhage, cardiovascular collapse, renal failure, seizures, encephalopathy and rhabdomyolysis may occur.

Other symptoms:

- Facial and peripheral oedema, ventricular arrhythmias.
- Muscle cramps.
- Investigations may show anaemia, leucopenia, thrombocytopenia or evidence of intravascular haemolysis.
- Death may occur from cardio respiratory or hepato renal failure. The adult respiratory distress syndrome (ARDS) has been reported.
- Survivors of severe poisoning may develop a peripheral neuropathy and skin lesions typical of chronic arsenic poisoning.

Inhalation

Rhinitis, pharyngitis, laryngitis and tracheobronchitis may occur. In severe poisoning tracheal and bronchial haemorrhage may occur.

CHRONIC TOXICITY

May occur following ingestion, inhalation or topical exposure. Features include weakness, lethargy gastrointestinal upset, dermal manifestations (hyperkeratosis and "raindrop" pigmentation of the skin), a peripheral (motor and sensory) neuropathy and psychological impairment. And also Peripheral vascular disease (cold sensitivity progressing to ulceration and gangrene), renal tubular or cortical damage and haematological abnormalities (notably pancytopenia) are reported.

MANAGEMENT

Ingestion

Very small ingestions:

1. Gastrointestinal decontamination is unnecessary.
2. Symptomatic treatment.

Substantial ingestions:

- Gastric lavage should be considered only if the patient present within one hour.
- Supportive measures are paramount. Intensive resuscitation may be required.
- Ensure adequate fluid replacement and close observation of vital signs including cardiac monitoring.
- Diarrhoea can be controlled with loperamide.
- Monitor blood urea, creatinine, electrolytes, liver function and full blood count.
- Collect blood and urine for arsenic concentration measurements.
- Isoprenaline is effective with phenytoin, lignocaine or propranolol as alternatives.

Antidotes - chelation therapy with either dimercaprol, DMSA or DMPS.

3.5 MANOSILAI

(Arsenic disulphidum, Red orpiment)

Introduction:

Manosilai is two types – one is piravi sarakku and the other one is vaippu sarakku. Vaippu sarakku is obtained by adding five parts of white arsenic with three parts of ganthagam. This is effective in skin leprosy, fever with chills, eye diseases, urinary tract infections, kapha diseases.

கொடிய குஷ்டம் காய்ச்சல் நடுக்கலஜ கல்லியிரைப்
புச்சிலந்திப் பேசறும னோசிலைக்குப் பேசு.

(Gunapadam, Thathu-Jeeva vaguppu)

It is also useful in the treatment of ajakallika (a type of boil occurring in children).

Purification methods:

- Manosilai is triturated with any one of the following – Ginger juice, Lemon juice, Cow's butter milk for three hours. Then it is dried to get purified form.
- The raw material is made into small pieces and kept soaked in 175 gm of fermented buttermilk in a clay vessel. It is kept in sunlight and mixed frequently.
- Manosilai is powdered and triturated with filtered water of lime and with kollu(Macrotyloma uniflorum) saaru.

Medicinal Uses:

Manosilai is mostly used not as such alone but in combination with other medicines as pills and oil. The oil is effective in the treatment of pauthiram(fistula) when applied topically. The pill is effective in the treatment of fever with chills when given orally.

It is mixed with ash of Nayuruvi (Achyranthes aspera) and applied externally for venkuttam.

Some preparations with Manosilai:

Medicines for eye diseases in **Sarabaenthirar nayana rogha sikichai**

1. Santhirodhaya Kuzhambu
2. Sarasaanjanam
3. Logaanjanam

Manosilai containing drugs in **Pulipani Marunthugal**

1. Rathinathi kuligai
2. Navarasa mezhugu

In **Agathiyar vaithiya surukkam,**

1. Kalakandamega naarayana chenduram
2. Poorna chanthirothayam

Manosilai drugs in **Vida vaithiya aarudam**

1. Thirugu thylum
2. Vida marunthugal

RED ORPIMENT

Introduction:

Arsenic disulphide is a naturally occurring form of arsenic and is found as realgar, one of the major arsenic containing mineral. Arsenic disulphide is insoluble in water and so poorly absorbed. It therefore represents a much less acute toxic hazard than soluble arsenic compounds.

Synonyms:

Red arsenic sulphide, Arsenic sulfide, Arsenic sulphide, Arsenic disulphide, Red orpiment, Ruby arsenic, Realgar.

Chemical name and formula:

Name : Arsenic di sulphide

Molecular formula: As_2S_4

Physical properties:

Colour	:	Red-brown
Melting point	:	320° C
Boiling point	:	565º C
Solubility	:	Insoluble in water
Molecular weight	:	213.97

Preparation

It is artificially prepared by fusing arsenious acid 5 parts and sulphur 3 parts.

Uses

Used in Leather industry, depilatory agent, paint pigment, shoe manufacture, pyrotechnics, rodenticide,(Factsheet for Realgar) colouring agent in fireworks.

As_2S_3 and As_4S_4 have been investigated as treatments for acute promyelocytic leukemia (APL).(D.-P. Lu,et,al)

Traditional uses

- Realgar, orpiment, and arsenopyrite provide nearly all the world's supply of arsenic as a byproduct of smelting concentrates derived from these ores.
- Realgar is poisonous. The ancient Greeks, who called it "sandaracha", knew that it was poisonous. It was used to poison rats in medieval Spain and in 16th century England.(In French)
- It is still sometimes used to kill weeds, insects, and rodents, even though more effective arsenic-based agents are available.
- It was, along with orpiment, a significant item of trade in the ancient Roman Empire and was used as a red paint pigment (Boston) and a medicine.
- Other traditional uses include manufacturing shot, printing and dyeing calico.

- It is purified by being rubbed with the juice of lemon or of Ginger. It is used as an alternative, febrifuge, and tonic, given in fever, cough, and asthma. In skin diseases it is used externally and locally it is applied to fistulous sores.

TOXICOKINETICS

Absorption

Arsenic disulphide is poorly absorbed after ingestion. The efficiency of absorption is dependent on particle size. Following inhalation irrespirable particles are trapped in the upper airways and deposited in the gastrointestinal tract by mucociliary clearance. Direct evidence of transcutaneous arsenical absorption in man is scarce.

Distribution

Absorbed arsenic is distributed to all body tissues. High concentrations would be expected in keratin-rich tissues such as hair, skin and nails due to sulphydryl group binding. Trivalent arsenic is methylated in the liver to methylarsonic acid and dimethyl arsenic acid Short-term studies on humans indicate that daily intake in excess of 0.5 mg progressively, but not completely, saturate the capacity to methylate inorganic arsenic.

Excretion

The half-life of arsenic in blood is about 60 hours with rapid renal excretion predominantly as mono- and dimethyl- derivatives.

Toxicity

Arsenic disulphide poisoning is rare. Exposure may occur via ingestion of herbal remedies or in industry.

ACUTE TOXICITY:

Dermal exposure:

Erythema, burning and itching, eczematous eruptions and folliculitis are typical.

Ocular exposure:

Arsenic disulphide is an eye irritant. Most injuries result from exposure to dusts, causing conjunctivitis, lacrimation, photophobia.

Ingestion:

Insoluble, poorly absorbed compounds such as arsenic disulphide represent less of a toxic hazard than water soluble arsenic species. However, substantial ingestions may produce serious systemic toxicity.

Gastrointestinal toxicity:

Nausea, vomiting, abdominal pain and diarrhoea are likely after substantial arsenic disulphide ingestion. Other features include burning of the mouth and throat with dysphagia and hyper salivation. Gastrointestinal haemorrhage may lead to cardiovascular collapse.

Nephrotoxicity:

Hypotension or rhabdomyolysis following substantial arsenic disulphide ingestion may precipitate renal failure.

Cardiovascular toxicity:

Tachycardia is typical in cases of arsenic intoxication and is contributed to by anxiety, intravascular fluid depletion and possibly a direct cardio toxic effect.

Neurotoxicity:

Acute substantial arsenical ingestion has caused muscle cramps, hearing deficit, encephalopathy and seizures.

Haemotoxicity:

In moderate or severe arsenic poisoning investigations typically shows anaemia, leucopenia or pancytopenia

Multi-organ toxicity:

Severe acute arsenic poisoning may result in death from cardio respiratory or hepato renal failure.

CHRONIC TOXICITY

May occur following ingestion, inhalation or topical exposure. Features include weakness, lethargy gastrointestinal upset, dermal manifestations (hyperkeratosis and "raindrop" pigmentation of the skin), a peripheral (motor and sensory) neuropathy and psychological impairment. And also peripheral vascular disease (cold sensitivity progressing to ulceration and gangrene), renal tubular or cortical damage and haematological abnormalities (notably pancytopenia) are reported.

MANAGEMENT

- Gastrointestinal decontamination is unnecessary.
- Symptomatic treatment.
- Supportive measures are paramount. Intensive resuscitation may be required. Ensure adequate fluid replacement and close observation of vital signs including cardiac monitoring.

3.6 VELLAI PAADANAM

(White arsenic)

Introduction:

This is obtained from nature in the land. It is mostly available in the combination with other metals such as sulphur, iron, copper etc. When used in small doses it stimulates appetite; improves body strength; reduces fever. It also stimulates the heart, lungs, bowels and reproductive organs.

General properties of Vellai Paadanam:

வெள்ளைப்பா டாணம் விடங்கடிதீ ரும்பூசக்
கொள்ளைச் சுரத்தோஷங் கோரசந்நி - தொள்ளையுறு
நாசிப்புண் வாய்ப்புண் நனைகிரந்தி போமுண்ண
ஆசிக்குங் கும்பமுலை ஆய்.

(Gunapaadam, Thathu-Jeeva vaguppu)

It has been found to be effective in the treatment of epidemic fever, poisonous bite, delirium, infections, ulcers of the nose and mouth and venereal ulcers. Further, this is also effective in filarial fever, skin diseases, etc.

Purification methods:

1. Vellai Paadanam (35 gm) is powdered and triturated with lemon juice. It is made into small cakes and dried. This process is repeated for seven times.
2. Vellai Paadanam is buried within the limestone and processed with the palm toddy for seven times to get purified.
3. Vellai Paadanam is subjected to churukku by adding avuri(Indigafera tinctoria) juice and white variety of erukku (Calotropis gigantean)juice for nine hours separately.
4. Strebulus asper pit oil (kutti palaa kuzhi thailam) is added gradually to the heated Vellai Paadanam to get purified form.

Toxic symptoms of Vellai Paadanam:

White arsenic is quite toxic when used even in slightly excess doses. When used in high doses it may cause death. Even when used in small doses for long period, it may produce toxic signs and symptoms.

Acute Poisoning:

Signs and symptoms are blisters, pain in the fingers of hands and legs, swelling of the face, ulcer of the upper lip, bad odour in the vomiting, pain in the throat, tasteless in the mouth, burning sensation in the stomach, bleeding diarrhoea and vomiting, excessive sweating, convulsion, impairment of memory, etc.

Chronic Poisoning:

The signs and symptoms of chronic poisoning are pruritis, eczematic lesions, kaamalai(liver damage causing jaundice), indigestion.

Antidotes for Vellai Paadanam:

- Four grams - in each of Cardamom (Elakkai) and Mukia Scaberilla *(Musumusukai)* root is boiled in water and of white sugar and Alum (Padikaaram) is mixed with the above decoction and is given twice for 40 days both morning and evening in empty stomach.

- Indigofera tinctoria *(Avuri)* root and black pepper is well boiled and the decoction is given.
- Indigofera tinctoria *(Avuri)* root is made into karkam and is given as Kottai pakalavu for two times a day.

ARSENIC TRIOXIDE

Introduction:

Arsenic trioxide is a white or transparent solid in the form of glassy, shapeless lumps or a crystalline powder that resembles sugar. It has no odour or taste. It forms readily when elemental metallic arsenic is heated to high temperatures or burned. When arsenic trioxide is burned, it releases toxic fumes and arsine gas which is highly toxic.

General Description:

Molecular formula	: As_2O_3
Molar mass	: 197.841 g/mol
Appearance	: White solid
Density	: 3.74 g/cm^3
Melting point	: 312.2 °C, 585 K, 594 °F
Boiling point	: 465 °C, 738 K, 869 °F
Solubility in water	: 20g/L(25 °C)
Solubility	: soluble in dilute acids and alkalies, practically insoluble in organic solvents (Patnaik, P.)

Natural occurrence:

Two minerals are known to possess the As_2O_3 chemical formula: arsenolite(regular) and claudetite (monoclinic). Both are relatively rare secondary minerals found in oxidation zones of As-rich ore deposits Traces of arsenic may naturally contaminate drinking water wells.

Medical applications:

- Despite the well known toxicity of arsenic, arsenic trioxide has long been of biomedical interest, dating to traditional medicines, where it is known as PiShuang in Chinese medicine and is still used to treat cancer and other conditions, (Gielen, M.; Tiekink, E. R.) and to homeopathy, where it is called arsenicum album.

- Some discredited patent medicines, e.g., Fowler's solution, contained derivatives of arsenic oxide.
- Arsenic trioxide under the trade name Trisenox is a chemotheraputic agent of idiopathic function used to treat leukemia that is unresponsive to "first line" agents. It is suspected that arsenic trioxide induces cancer cells to undergo apoptosis.
- Due to the toxic nature of arsenic, this drug carries significant risks. Use as a cytostatic in the treatment of refractory promyelocytic (M3) subtype of acute myeloid leukemia (Soignet, S. L.).
- The combination therapy of arsenic trioxide and all-trans retinoic acid (ATRA) has been approved by the U.S. Food and Drug Administration (FDA) for treatment of certain leukemias. University of Hong Kong developed a liquid form of arsenic trioxide that can be administered orally.
- Arsenic trioxide also appears to be a promising therapeutic agent for autoimmune diseases. (Bobé, P.)
- The enzyme thioredoxin reductase has recently been identified as a target for arsenic trioxide. (Lu, J.; Chew, E. H.)
- Arsenic trioxide in combination with ascorbic acid and buthionine sulfoxide decrease intracellular glutathione to a greater extent, and render malignant cells more sensitive to apoptosis.
- Arsenic trioxide induced apoptosis was not enhanced by ascorbic acid in normal cells, suggesting that this combination may be selectively toxic to some malignant cells.

Toxicology:

Arsenic trioxide is readily absorbed by the digestive system: toxic effects are also well known upon inhalation or upon skin contact. Elimination is rapid at first (half-life of 1–2 days), by methylation to monomethylarsonic acid and dimethylarsonic acid, and excretion in the urine, but a certain amount (30–40% in the case of repeated exposure) is incorporated into the bones, muscles, skin, hair and nails (all tissues rich in keratin) and eliminated over a period of weeks or months.

Acute arsenic poisoning:

- The first symptoms of acute arsenic poisoning by ingestion are digestive problems: vomiting, abdominal pains, diarrhea often accompanied by bleeding.
- Sub-lethal doses can lead to convulsions, cardiovascular problems, inflammation of the liver and kidneys and abnormalities in the coagulation of the blood. These are followed by the appearance of characteristic white lines (Mees stripes) on the nails and by hair loss.
- Lower doses lead to liver and kidney problems and to changes in the pigmentation of the skin. Even dilute solutions of arsenic trioxide are dangerous on contact with the eyes. The poisonous properties are legendary and the subject of an extensive literature. (Emsley, J.)

Chronic arsenic poisoning:

- Chronic arsenic poisoning is known as arsenicosis. This disorder affects workers in smelters, in populations whose drinking water contains high levels of arsenic (0.3–0.4 ppm), and in patients treated for long periods with arsenic-based pharmaceuticals.
- Long-term ingestion of arsenic trioxide either in drinking water or as a medical treatment can lead to skin cancer.
- In Austria, there lived the so-called "arsenic eaters of Styria", who ingested doses far beyond the lethal dose of arsenic trioxide without any apparent harm. Arsenic is thought to enable strenuous work at high altitudes, e.g. in the Alps. (Whorton, J. C.)
- Reproductive and Developmental Effects - Arsenic ions released from arsenic trioxide within the body can cross the placenta and affect the developing fetus arsenic is also excreted in breast milk. Experimental animal studies support an association between high ingested arsenic dose and fetal toxicity.

Treatment:

- There is no specific antidote for arsenic trioxide.
- Symptematic treatment
- Prehospital treatment consists of supportive care and gastric decontamination.
- Chelation therapy is strongly recommended.
- In cases of ingestion, emesis should not be induced.
- Aggressive decontamination with gastric lavage is recommended within 1 hour of ingestion of a life-threatening amount of poison.

3.7 POORAM

(Rasa karpooram, Hydrargyrum subchloride, Calomel)

Introduction:

Although the Pooram does not find a place in the list of sixty four paadanam it is considered as one among them by the medical practitioners. It is prepared by the combination of Rasam and Salt. It has laxative, tonic, antiseptic and diuretic properties. It induces biliary secretion.

General properties of Pooram:

சசிவன்ன கருப்பூ ரத்தில் சாதித்த கயஞ்சு வாசம்
பசிகலி தாப சோபம் பவுத்திரம் பிளவை குஷ்டம்
வசிதரு கிராணி யோடு வளரதி சார மேகம்
இசிதரு மிசிவு சூலை யிவைபல ரோகம் போமே.

(Gunapaadam, Thathu – Jeeva vaguppu)

When the Pooram is taken along with jaggery for seven days, it cures various types of excruciating pains, throbbing pain in the lumbar region, burning sensation, ulcer due to disorders of vatham, manjal kaamalai(Jaundice), pyrexia, chronic ulcers, venereal diseases, indigestion, vomiting, diarrhoea, worm infestation, rheumatism, itching, constipation, scabies etc.

Purification methods:

1. Pooram(35gm) is processed by surukku method with mother's milk for three hours and with garlic thylam for nine hours. It is taken out as purified.
2. Before adding the Pooram in lekeyams, it is subjected to surukku process with the juice of musumusukkai(Mukia maderaspatana).

Pooram does not dissolve in water rapidly. If it is consumed in large quantity, it manifests poisonous effects.

The signs and symptoms of Calomel poisoning are multiple red boils may appear on the face, acne formation, ulcers in the chest, mouth and tongue, diarrhoea and dysentry, scrotal swelling and ulcer in the uvula.

Antidote for poisoning:

பகருவாய் பூரத்தைப் பாங்குடன்றின் றோர்கட்
கிகலரிய குல்லையின்சா றீக - தகவுடைய
ஏரண்ட நெய்யா மெழிலவுரி வேராகும்
சீர்கொண்ட பாகலிலை தேர்.

(Agathiyar vida pirathi vida thirattu)

1. Ocimum sanctum *(Thulasi)* leaf juice, or Castor oil or Momordica charantia (*Kombu Pagal)* leaf juice, twice a day till the poisonous effects are neutralized. Again a small spoon of Castor oil may be given when it is given second time.

2. Indigofera tinctoria *(Avuri)* root bark is ground in hot water and is given in the quantity of a Sundai *(Solanum pubescens)* twice daily.

3. To reduce the poisonous effect, the powder of Alum *(Seenakkaram)* mixed in the tender coconut water is given. The kernel of the tender coconut is also given.

4. Nilappanai kizhangu kudineer:

    Black musali tubers (Curculigo orchides)
    Indian penny wort root (Centella asiatica root)
    Root of sessile plant (Alternanthera sessiles)
    Beetle killer (Clerodendrum serratum –Kandu paarangi)

All the above ingredients are mixed and boiled to make a decoction. This decoction is used twice a day for two or three weeks with suitable diet restrictions.

Some preparations containing Pooram:

1. Vaalai sancheevi mathirai

    Dosage : Uluthalavu.
    Therapeutic uses : Vatha roagam, Maantham, Suram.
    Reference: Siro rathina vaithiya poosanam, pg no: 49.

2. Mahasathuvaathi kuligai

    Dosage : Sundai alavu.
    Therapeutic uses : Gunmam, Peligai, Kudal vayu.
    Reference: Agathiyar vaithiya vallathi, pg no: 35.

3. Sootha vallathi urundai

Dosage : Milagalavu.

Therapeutic uses : Kiranthi, Kuttam, Puttru, Vippuruthi, Pilavai.

Reference: Agathiyar vaithiya vallathi, pg no: 20.

4. Poovarasankaai ennai

Dosage : 1/4 palam

Therapeutic uses : Korukku, Kiranthi, Karumegam.

Reference: Sikicha rathna deepam, pg no: 158.

5. Karpoora chinthamani maathirai

Dosage : 1/2 - 1 tablet.

Anupaanam : Sugar, Ghee.

Therapeutic uses : Soolai noi, Keel vayu.

Reference: Anuboga vaithiya navaneetham; part - 4, pg no: 60.

6. Gurumurai pathangam

Dosage : 1/2 -1 arisi eadai

Anupaanam : Palm jaggery, Butter.

Therapeutic uses : Kiranthi, Gunmam, Narambu vaatham,

Reference: Anuboga vaithiya navaneetham; part - 7, pg no: 19.

7. Rasakarpoora kattu

Therapeutic uses : Sanni, Suram, Naa varatchi.

Reference: Aathma ratchamirutham ennum vaithiya saara sagiragam, pg no: 149.

8. Sanni maathirai

Dosage : Kundriyalavu.

Anupaanam : Honey.

Therapeutic uses : Sanni.

Reference: Sootchuma vaithiyam – 200.

MERCUROUS CHLORIDE (CALOMEL)

INTRODUCTION

Mercurous chloride, mercury (I) chloride or calomel is a white crystalline powder and very slightly soluble in water. Mercurous chloride is a less dangerous poison than mercuric chloride because it is much less soluble; but it is highly toxic if retained in the body. It is also found in nature as horn quicksilver. It was used medicinally as a purgative, cathartic, liver stimulant, and to eliminate parasitic worms, but is rarely so used today because it is readily decomposed into metallic mercury and the very poisonous mercuric chloride found on exposure to sunlight or if heated in the presence of moisture.

COMPOSITION Mercurous chloride (85.0% Hg, 15.0% Cl).

TESTS Completely volatilizes on charcoal, without melting.

DISTINGUISHING CHARACTERISTICS

The sectile character and the adamantine luster distinguish it from everything except from the silver halides. Silver minerals melt but do not volatilize completely on the charcoal, leaving a flattened silver residue. In a mercury association ore the fluorescence is significant.

CRYSTAL DESCRIPTION - Tetragonal -- Ditetragonal Bipyramidal

Usually found as crystals, often minute and coating other minerals. Most often tabular, sometimes pyramidal. Commonly in skeletal parallel growths rather than good individual crystals.

GENERAL PROPERTIES

Chemical formula: Hg_2Cl_2

Chemical name: Mercurous Chloride

Mineralogical name: CALOMEL

Color: White, Yellowish gray, Gray, Yellowish white, Brown

(Darkening on exposure to light).

Habit:	Earthy - Dull, clay-like texture with no visible crystalline affinities. Prismatic - Crystals Shaped like Slender Prisms (e.g. tourmaline).
Molar mass:	472.09 g/mol
Solubility in water:	0.2 mg/100 ml.
Solubility:	Insoluble in ethanol, ether
Luminescence:	Fluorescent, Short UV=dark red, Long UV=dark red.
Luster:	Adamantine - Resinous, translucent; fluorescent red.
Streak:	pale yellowish white
Hardness:	1.5-2 - Talc-Gypsum
Specific gravity:	6.5
Density:	6.4 - 6.5, Average = 6.45
Melting point:	525 °C (triple point)
Boiling point:	383 °C (sublimes)
Diaphaneity:	Translucent to sub translucent
Fracture:	Sectile - Curved shavings or scrapings produced by a knife blade, fracture conchoidal (e.g. graphite)

Refractive index (nD) - 1.973

Highly reactive with metals except Sn, Pb, Ag, Au

STRUCTURAL PROPERTIES

Mercury is unique among the group of 12 metals for its ability to form the M –M bond so readily. Hg_2Cl_2 is a linear molecule. The crystal structure is unit cell distorted octahedral coordination of mercury. The chemical bonding are

The Hg–Hg bond length of 253 pm (Hg – Hg in the metal is 300 pm) and the Hg – Cl bond length in the linear Hg_2Cl_2 unit is 243 pm.

The overall coordination of each Hg atom is octahedral as, in addition to the two nearest neighbors, there are four other Cl atoms at 321 pm.

PREPARATION

Mercurous chloride is prepared by sublimation from a mixture of mercury and mercuric chloride or by precipitation from a mercurous chloride solution on adding chloride ion. . It may be obtained by heating mercury in chlorine, or by reducing mercuric chloride (corrosive sublimate) with mercury or sulphuric acid. It is manufactured by heating a mixture of mercurous sulphate and common salt in iron retorts, and condensing the sublimed calomel in brick chambers. In the wet way it is obtained by precipitating a mercurous salt with hydrochloric acid. Long continued boiling with water gives mercury and mercuric chloride; dilute hydrochloric acid or solutions of alkaline chlorides convert it into mercuric chloride on long boiling.

CHEMICAL REACTIONS

Mercurous chloride forms by the reaction of elemental mercury and mercuric chloride

$$Hg + HgCl_2 \rightarrow Hg_2Cl_2$$

It can be prepared via metathesis reaction involving aqueous mercury (I) nitrate using various chloride sources including NaCl or HCl.

$$2HCl + Hg_2(NO_3)_2 \rightarrow Hg_2Cl_2 + 2HNO_3$$

Ammonia causes Hg2Cl2 to disproportionate:

$$Hg_2Cl_2 + 2NH_3 \rightarrow Hg + Hg\,(NH_2)\,Cl + NH_4Cl$$

REACTIVITY PROFILE

Mercurous Chloride is incompatible with acetylene, ammonia, chlorine dioxide, azides, calcium (amalgam formation), sodium carbide, lithium rubidium and copper.

HEALTH HAZARD

Acute poisoning can result from inhaling dust concentrations of 1.2-8.5 mg/m 3 in air; symptoms include pain and tightness in chest, coughing, and difficulty in breathing. Compound is an irritant, cathartic, or purgative; rarely, **"calomel sickness,"** a benign reaction with fever and rash, appears after about 1 week; seldom causes systemic poisoning but may be fatal if retained to 30-40 mg/kg. Contact with eyes causes mild irritation.

EMERGENCY

Eye contact: Immediately flush the eye with water. If irritation persists, go for medical help.

Skin contact: Wash off with soap and water.

Do not discard into a sink or into normal solid waste containers.

Wear Protective equipment, safety glasses, and gloves.

TOXICOLOGICAL ASPECT

May be fatal if swallowed or inhaled. Chronic exposure may lead to systemic effects and build-up of mercury in the brain, liver and kidneys. May cause memory loss, tremors and other serious effects.

LD_{50} - 210 mg/ kg.b.wt on oral administration to rat.(Thomas W. Clarkson)

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY	**CALOMEL**	**MERCURY**
CA Prop 65 Developmental Toxin	Yes	Yes

Symptoms of Calomel Exposure from the International Chemical Safety Cards (ICSC) (Thomas W. Clarkson)

ROUTE OF EXPOSURE	SYMPTOMS	FIRST AID
Inhalation	Cough. Sore throat.	Fresh air rest. Refer for medical attention.
Skin	May be absorbed. Redness may develop.	Remove contaminated clothes. Rinse skin with plenty of water or shower. Refer for medical attention.
Eyes	Redness may be developed.	First rinses with plenty of water for several minutes (remove contact lenses if easily possible) then take to a doctor.
Ingestion	Abdominal pain. Diarrhoea. Vomiting. Metallic taste.	Rinse mouth. Induce vomiting (ONLY IN CONSCIOUS PERSONS). Refer for medical attention.

AQUATIC ECOTOXICITY

All Toxic Effects for Organism Group

ORGANISM GROUP	EFFECTS NOTED
Aquatic Plants	Cell(s)
Echinoderms	Development
Fish	Mortality

Insects	Intoxication
Mollusks	Accumulation, Biochemistry
Nematodes and Flatworms	Development, Mortality
Zooplankton	Mortality

ORGANISM GROUP	**AVERAGE ACUTE TOXICITY**
Fish	Moderately Toxic
Nematodes and Flatworms	Slightly Toxic
Zooplankton	Very Highly Toxic

3.8 KAANTHAM

(Magnetic oxide of iron)

Introduction:

There are different varieties of magnet, such as, Kal kaantham, Oosi kaantham, Pachai kaantham, Arakku kaantham, Mayir kaatham, Urulai kaantham, Palagai kaantham and thagattu kaantham. In general, the magnetic oxide of iron has got the properties as iron. However, it is considered that the magnetic oxide of iron is superior to iron in many aspects.

காந்தத்தாற் சோபைகுன்மங் காமிலமே கம்பாண்டு
சேர்ந்ததிரி தோடவெட்டை சீதங்கால் - ஒய்ந்தபசி
பேருதரங் கண்ணோய் பிரமியநீ ராமையும்போம்
ஓரினிரை யாயுளும் உன்.

(Gunapaadam, Thathu-Jeeva vaguppu)

This is very effective in the treatment of swelling, ulcer(gunmam), jaundice (kaamaalai), venereal diseases of three humours, leucorrhoea(vellai), kapha vadha diseases, dyspepsia(mantham), anasarca (mahodaram), eye diseases, gonorrhoea(brahmiyam), and spleenomegaly. It also increases longevity.

Purification and detoxification:

1. Magnetic oxide of iron 35 gm
   Root bark juice of Tanner's cassia 210gm
   (Cassia auriculata)- Ponnavarai

Magnet is soaked in root juice of Tanner's cassia and is kept in sunlight from morning to evening for ten days. Then it is dried for two days without adding the juice. This process is repeated twice and washed to obtain purified and detoxified magnet. The medicine manufactured from this magnetic oxide destroys diseases of vatha and protects life.

2. Magnetic oxide of iron 35gm
   Juice of Kondrai (Cassia fistula) 280gm

Basil (Ocimum sanctum) 280gm

Indian pennywort (Centella asiatica) 280gm

Magnet is soaked in Indian laburnum (Cassia fistula) and kept in sunlight for nine days. (Daily fresh juice should be used). Then it is dried without adding the juice. This process is repeated with the leaves juice of basil (Ocimum sanctum) and Indian pennywort (Centella asiatica). After twenty seven days, the purified form of magnetic oxide of iron is obtained.

3. The magnetic oxide iron is powdered and wrapped in a cloth. It is boiled with Kaadi (vinegar steam) and with the steam of horse gram decoction then it is boiled, washed and dried.

4. The magnetic oxide of iron is soaked in lemon juice, sour butter milk for three days in each. It is then dried in sunshine, and washed. Thus it is purified form is obtained.

5. The magnetic oxide of iron is heated in a furnace and dipped in horse gram decoction for 7 to 21 times to get it in purified form.

Antidote for metal poisoning:

The toxic symptoms may be occur with metal containing preparations (without iron). For this, Aya chenduram or Aya parpam or iron mixed tablets is used as antidote.

MAGNETIC OXIDE OF IRON

Introduction:

Magnetite is a mineral, one of the two common naturally occurring **oxides of Iron (chemical formula Fe_3O_4)** and a member of the spinel group. Magnetite is the most magnetic of all the naturally occurring minerals on Earth. Naturally magnetized pieces of magnetite, called lodestone, will attract small pieces of iron, and this was how ancient people first noticed the property of magnetism.

General decription:

Category : Oxide minerals – Spinel group

Formula : Iron(II,III) oxide, $Fe^{2+}Fe^{3+}{}_2O_4$

Colour : Black, gray with brownish tint in reflected sun

Crystal habit	: Octahedral, fine granular to massive
Crystal system	: Isometric Hexoctahedral
Tenacity	: Brittle
Luster	: Metallic
Streak	: Black [1]
Specific gravity	: 5.17–5.18
Solubility	: Dissolves slowly in hydrochloric acid

Properties:

- Lodestones were used as an early form of magnetic compass. Magnetite typically carries the dominant magnetic signature in rocks, and so it has been a critical tool in paleomagnetism, a science important in discovering and understanding plate tectonics and as historic data for magnetohydrodynamics and other scientific fields.
- Magnetiteand other heavy minerals (dark) in a quartz beach sand (Chennai, India).
- Magnetite is sometimes found in large quantities in beach sand. Such black sands (mineral sands or iron sands) are found in various places, such as California and the west coast of the North Island of New Zealand. The magnetite is carried to the beach via rivers from erosion and is concentrated via wave action and currents.(Templeton.)
- Huge deposits have been found in banded iron formations. These sedimentary rocks have been used to infer changes in the oxygen content of the atmosphere of the Earth.

Transformation of ferrous hydroxide into magnetite:

Under anaerobic conditions, the ferrous hydroxide ($Fe(OH)_2$) can be oxidized by the protons of water to form magnetite and molecularhydrogen. This process is described by the Schikorr reaction:

$3\ Fe(OH)_2 \rightarrow Fe_3O_4 + H_2 + 2\ H_2O$

Ferrous hydroxide → magnetite + hydrogen + water

The well-crystallized magnetite (Fe_3O_4) is thermodynamically more stable than the ferrous hydroxide ($Fe(OH)_2$).

Biological occurrences:

- Biomagnetism is usually related to the presence of crystals of magnetite, which occurs widely in organisms. (H. A. Lowenstam)
- These organisms range from bacteria (e.g., Magnetospirillum magnetotacticum) to several animals, where these crystals are found in the brain. (Massart, R)
- These crystals are thought to be involved in magnetoreception, the ability to sense the polarity or the inclination of the Earth'smagnetic field, and to be aid in navigation.

Catalysis:

Magnetite is the catalyst for the industrial synthesis of ammonia.

As a sorbent

- Magnetite powder efficiently removes arsenic(III) and arsenic(V) from water, the efficiency of which increases ~200 times when the magnetite particle size decreases from 300 to 12 nm.
- Arsenic-contaminated drinking water is a major problem around the world, which can be solved using magnetite as a sorbent.

Other:

- Because of its stability at high temperatures, it is used for coating industrial watertube steam boilers.
- The magnetite layer is formed after a chemical treatment (e.g. by using hydrazine).

3.9 SAATHIKAI

(Common Nutmeg, Myristica fragrens Houtt)

Introduction:

It is a fragrant fruit cultivated in the East India, Ceylon, Malabar, Nilgris and other places. The outer portion which forms the kernal of the fruit is yellowish, the seed is within this. The aromatic veins coated over the seed are known as mace (Saathipathiri).

Saathikkai is also called as Kulakkai.

Parts used:

Seeds.

Action:

Stimulant	Carminative
Narkotic	Aromatic
Aphrodisiac	Tonic

General properties:

தாது நட்டம் பேதி சருவாசி யஞ்சிர நோய்
ஓதுசுவா சங்காசம் உட்கிரணி - வேதோ
டிலக்காய் வரும்பிணிபோம் ஏற்றமயல் பித்தங்
குலக்கா யருந்துவர்க்குக் கூறு.

(Gunapaadam – Mooligai Vaguppu)

In Siddha medicines, it is useful in cases of spermatorrhoea or loss of semen, diarrhoea due to the derangement of vatham.

The expressed oil is useful application for ulcers, sores, wounds, etc, and also serves in cases of rheumatism, paralysis, sprains, etc.The oil is applied with advantage for tooth ache. It is helpful in atonic diarrhoea, dysentery and dyspepsia.

The powder form of saathikkai is prescribed for quenching the thirst of cholera patients. Externally, it is applied around the eyes for getting bright eyes.

Scientific classification:

Kingdom	: Plantae
Order	: Magnoliales
Family	: Myristicaceae
Genus	: **Myristica**
Species	: fragrens

The nutmeg tree is any of several species of trees in genus Myristica. The most important commercial species is Myristica fragrans, an evergreen tree is important for two spices derived from the fruit: nutmeg and mace(Encyclo)

Essential oils:

The essential oil obtained by steam distillation of ground nutmeg is used widely in the perfumery and pharmaceutical industries. This volatile fraction typically contains 60-80% d-camphene by weight, as well as quantities of d-pinene, limonene, d-borneol, l-terpineol, geraniol, safrol, and myristicin (Merck Index).

The oil is colourless or light yellow, and smells and tastes of nutmeg. It contains numerous components of interest to the oleochemical industry, and is used as natural food flavouring in baked goods, syrups, beverages, and sweets. The essential oil is also used in the cosmetic and pharmaceutical industries, for instance, in toothpaste, and as a major ingredient in some cough syrups. In traditional medicine, nutmeg and nutmeg oil were used for disorders related to the nervous and digestive systems.

After extraction of the essential oil, the remaining seed, containing much less flavour, is called "spent". Spent is often mixed in industrial mills with pure nutmeg to facilitate the milling process, as nutmeg is not easy to mill due to the high percentage of oil in the pure seed.

Nutmeg butter:

Nutmeg butter is obtained from the nut by expression. It is semi-solid, reddish brown in colour, and tastes and smells of nutmeg. Approximately 75% (by weight) of

nutmeg butter is trimyristin, which can be turned into myristic acid, a 14-carbon fatty acid, which can be used as a replacement for cocoa butter, can be mixed with other fats like cottonseed oil or palm oil, and has applications as an industrial lubricant.

Common uses:

- Nutmeg and mace have similar sensory qualities, with nutmeg having a slightly sweeter and mace a more delicate flavour.
- In Indian cuisine, nutmeg is used in many sweet as well as savoury dishes (predominantly in Mughlai cuisine). It is also added in small quantities as a medicine for infants. It may also be used in small quantities in garam masala. Ground nutmeg is also smoked in India.
- In originally European cuisine, nutmeg and mace are used especially in potato dishes and in processed meat products; they are also used in soups, sauces, and baked goods. In Dutch cuisine, nutmeg is added to vegetables such as Brussels sprouts, cauliflower, and string beans.
- Japanese varieties of curry powder include nutmeg as an ingredient.

Medicinal:

Used as an anti-diarrhoea agent for patients with medullary carcinoma of the thyroid. The effectivenes of the treatment may be due to the inhibition of prostaglandin synthesis in the mucosa and submucosa of the colon. The dosage given was 9 tablespoons orally per day but it may vary between patients to avoid toxic symptoms.

High risk circumstances: Abuse

Nutmeg has been known for its hallucinogenic properties for a long time. Adults may abuse the hallucinogenic properties of nutmeg. Children may be at high risk at home, since nutmeg may be widely available as a cooking additive. In the course of its use in traditional medicine, overdose may occur.

Medical research:

Nutmeg has been used in medicine since at least the seventh century. In the 19th century it was used as an abortifacient, which led to numerous recorded cases of nutmeg

poisoning. Although used as a folk treatment for other ailments, unprocessed nutmeg has no proven medicinal value today (Shafer, Jack).

One study has shown that the compound macelignan isolated from Myristica fragrans may exert antimicrobial activity against Streptococcus mutans(Devi, P. B.).

A methanolic extract from the same plant inhibited Jurkat cell activity in human leukemia, but these are not currently used treatments.

TOXICOLOGICAL ASPECT

Poisonous parts:

The seeds (nutmeg) and, to a lesser extent, the aril (mace).

Main toxins:

Myristicin

elemicin, but intoxication is not thought to be due to these alone.

Main risks and target organs:

- transient psychosis
- possibility of fatty liver and hepatic necrosis
- transient renal toxicity
- possible carcinogen and teratogen
- possibility of death occurring

CLINICAL EFFECTS

Acute poisoning:

In the toxic state, the patient first feels excited and may have psychedelic hallucinations. This is followed by a period of drowsiness, delirium and, possibly, unconsciousness. Thirst has been reported. Mental concentration may either be impaired or enhanced; delirium with agitation, disorientation and incoherence may develop. Prison inmates taking nutmeg "trips" have compared it to alcohol, heroin and "high", relaxed and drowsy. Some reported a sleepy feeling, others, restlessness and tense. Most patients with accidental nutmeg intoxication experience an impending sense of doom after the

initial excitation. The effects of nutmeg are most often compared to those of marihuana. Although the hallucinogenic effects of nutmeg are satisfactory, the side effects often discourage its use as such an agent.

Chronic poisoning

Chronic poisoning by oral administration has caused temporary psychosis in prison inmates (up to six months).

Course, prognosis, cause of death:

Not all the symptoms listed below appear in every case poisoning. Contradicting symptoms may occur at different times during the course of intoxication. The subject initially feels excited, then drowsy before a delirious state sets in. This is followed by a deep sleep. During this period, cyanosis of the extremities and convulsions may occur. Generally there is tachycardia and an increase in blood pressure.

Acidosis may set in because of diarrhoea and vomiting which is usually present along with various other gastrointestinal symptoms such as abdominal cramps. The subject may or may not be hallucinating but usually expresses a feeling of impending doom. Nutmeg intoxication usually clears by itself within 24 hours, however, it has been reported that psychosis may set in. Transient renal toxicity has also been reported causing an increase in albumin and non-protein nitrogen content in the urine, returning to normal within 24 hours.

Systematic description of clinical effects

Cardiovascular

- Tachycardia.
- Hypertension or hypotension may occur.
- Chest pains or tightness in chest.

Neurological

Central nervous system (CNS)

- Severe headaches.
- Drowsiness several hours after taking nutmeg.

- Fitful sleep/convulsions.
- Hallucinations (predominantly visual).
- Sedation.
- Euphoria.
- Concentration may be impaired or improved.
- Excitation resembling that caused by anticholinergic intoxication.
- Feeling of impending doom.

Sometimes unusual behaviour occurs during intoxication such as hysteria and wild trashing of limbs, and behaviour resembling that of a snarling dog.

Peripheral nervous system

- Initial stimulation after administration.
- Strong tingling in the fingers and toes shortly after snuffing some nutmeg.
- Numbness in hand and feet half an hour after snuffing nutmeg.
- Absent limb reflexes.

Autonomic nervous system

- Profuse sweating several hours after administration possibly reflecting amphetamine-type reaction.
- Absence of salivation.

Skeletal and smooth muscle

Muscular excitation several hours after administration.

Gastrointestinal

- Nausea.
- Vomiting.
- Diarrhoea.
- Abdominal pain.

Hepatic

- Hepatic necrosis in heavy poisoning.
- Fatty degradation of liver.

Renal

Transient renal toxicity producing albuminuria.

Non-protein nitrogen content in urine which returns to normal within 24 hours.

Summary of clinical effects:

Nutmeg intoxication resembles anticholinergic intoxication, e.g. profuse sweating, flushed face, delirium, dry throat etc. There is always an altered state of mind, e.g. hallucinations, confusion and an impending sense of doom. Clinical symptoms may be contradictory depending on the length of time lapsed after ingesting the toxin. Symptoms also vary according to the dose taken and the variability between different samples of nutmegs.

Psychoactivity and toxicity:

In low doses, nutmeg produces no noticeable physiological or neurological response, but in large doses, raw nutmeg has psychoactive effects. In its freshly-ground (from whole nutmegs) form, nutmeg contains myristicin, a monoamine oxidase inhibitor and psychoactive substance. Myristicin poisoning can induce convulsions, palpitations, nausea, eventual dehydration, and generalized body pain (Demetriades, A. K). It is also reputed to be a strong deliriant (Erowid).

In case reports, raw nutmeg produced anticholinergic-like symptoms, attributed to myristicin and elemicin (Weil, Andrew).

In case reports intoxications with nutmeg had effects that varied from person to person, but were often reported to be an excited and confused state with headaches, nausea and dizziness, dry mouth, bloodshot eyes and memory disturbances. Nutmeg was also reported to induce hallucinogenic effects, such as visual distortions and paranoid ideation. In the reports nutmeg intoxication took several hours before maximum effect was reached. Effects and after-effects lasted up to several days (Brenner, N).

Myristicin poisoning is potentially deadly to some pets and livestock, and may be caused by culinary quantities of nutmeg harmless to humans. For this reason, it is recommended not to feed eggnog to dogs.[30]

Toxicity during pregnancy:

Nutmeg was once considered an abortifacient, but may be safe for culinary use during pregnancy. However, it inhibits prostaglandin production and contains hallucinogens that may affect the fetus if consumed in large quantities (Herb and drug safety chart).

3.10 KUPPAIMAENI

(Acalypa indica)

Description:

Acalypha indica Linn is a member of the Euphobiaceae family. It is essentially a weed which grows in waste areas. It is an erect, annual herbaceous plant with numerous ascending branches. Flowers are unisexual. Fruit capsules are small, concealed by the bracts. The seeds are ovoid, smooth, pale brown in colour.

Arimanjari, Poonai vanangee, Maarjaala moghini are some other names.

Plant Part Used:

Whole plant

Action:

Anodyne	Anthelmintic
Cathartic	Diuretic
Emetic	Expectorant

General properties:

தந்தழு லப்பிணிதீத் தந்திடுபுண் சர்வவிடம்
உந்துகுன்மம் வாதம் உதிரழு- லந்தினவு
சூலஞ்சு வாசம் தொடர்பீ சங்கபம்போம்
ஞாலங்கொள் மேனியத னால்.

(Gunapaadam, Mooligai Vaguppu)

The leaves are very helpful in gum diseases, burnt ulcers and in plant toxins. It is also used in colic pain, itching, vatha diseases, pricking pain and kapha diseases.

It may be taken as decoction form or in powder form or as fresh extract.

Chemical Constituents:

Kaempferol glycosides, mauritianin, clitorin, nicotiflorin and biorobin, acalyphin, epiacalyphin, tannins, beta-sitosterol, acalyphamide, aurantiamide, succinimide, flindersin, triacetonamine, n-octacosanol, quebrachitol, hydrocyanic acid.

Traditional Use:

Tribes of Kerala, Rajasthan and Madhya Pradesh use fruits: in asthma, cough, bronchitis and earache; plant and fruit: as an expectorant, laxative, pneumonia and rheumatism; leaf: in skin diseases like scabies.

Acalypha indica is considered by the traditional practitioners to be bitter, acrid, expectorant, purgative, emetic, gastrointestinal irritant and diuretic. (P. K. Warrier)

The leaves are known to have laxative properties and are given in the form of powder or decoction by Indians to treat constipation. In cases of obstinate consitipation the leaves are grounded into paste and made into a ball and introduced per rectum. It is supposed to relax the sphincter ani and produces free motion. In Madagascar, the root decoction is used as a laxative. A. indica is also considered as a vermifuge in this case the root infusion is being used by people of Seychelles and Reunion Islands. On the other hand the Indians make use of the leaves together with garlic to expel the vermins.

The leaves are considered as an emetic and most practitioners make use of the leaf sap as part of a concoction to induce vomiting. It is considered as safe even for children.

Kupaimaeni is considered an expectorant and the juice expressed from the leaves is being given to treat croup, while in smaller doses it helps in productive cough by aiding its expectoration. The root infusion or decoction is given for asthma.

The leaves of Kupaimaeni has been advocated for use in the treatment of various parasitic skin conditions. One of the most common applications is the treatment of scabies.

East Africans use the leaf sap to treat eye infection while in Namibia the decoction is instilled into the eye for the same purpose. In India a decoction of the leaves is instilled into the ears to treat otalgia and otitis.

In Comoros, the leaf decoction is used in cream form to treat joint pains and rheumatism, while in India the leave juice mixed with oil is used instead. Poultice of the plant is applied to the head for headache. (Gabriëlla Harriët Schmelzer)

Pre-Clinical Data

Pharmacology

Postcoital infertility activity:

This effect is due to some estrogenic activity as evidenced by histological studies of the uterus. (Hiremath SP)

Wound healing activity:

Studies found that A. indica does have wound healing ability, however, it is inferior to Heliotropium indicum which has better activity and tensile strength.

Neutralisation potential of Viper venom activity:

The viper venom induced lethality by haemorrhage, necrotisation and mast cell degranulation in rats and cardiotoxicity and neurotoxicity in frogs. Preadministration of the ethanol leaf extracts to test animals was found to significantly inhibit these effects. It was found that the extract also inhibited venom-induced lipid peroxidation in RBC, decrease GSH and catalase levels in rat kidney tissue. This indicates that the ethanol leaf extract of Acalypha indica possesses potent snake venom neutralising properties. (Shirwaikar A, et al)

Antibacterial activity:

A study of the antibacterial activity of 4 different extracts (hexane, chloroform, ethyl acetate and methanol) from the leaves of A. indica was carried out against Gram positive (Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Streptococcus faecalis) and Gram negative (Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa) bacteria. All the extracts exhibited antibacterial activity against the Gram positive organisms with MIC 0.156 to 2.5 mg/mL while amongst the Gram negative bacteria only Pseudomonas aeruginosa was susceptible. (Govindarajan M)

Toxicities:

The raw herb is considered poisonous, emetic, and causes intestinal irritation. Pollens may cause allergy. (C. P. Khare, 2004)

COLLECTION, IDENTIFICATION, PURIFICATION AND PREPARATION OF PUTTRU PATHANGAM

COLLECTION OF DRUG:

The raw drugs were collected from country drug merchant shop, Thakkalai, Kanyakumari.

AUTHENTICATION:

The raw drugs were authenticated by Siddha Central Research Institute, Chennai. The plant drugs were authenticated by Department of Medicinal Botany, National Institute of Siddha.

PREPARATION : Gunapaadam Lab, National Institute of Siddha.

METHODS OF PURIFICATIONS:

Rasam

Mercury is mixed with the juice of whole plant of Thumbai (Leucas aspera) 4¾ palam (166.25gm) and insolated. This procedure is repeated for 10 days by adding fresh juice daily. Then it is insolated without adding the juice. This procedure is also repeated for one more day. Then the mercury and the powder present along with it are placed in a mud pot. The juice of Thumbai 2 padi (2800gm) is added, sealed and burried for 20 days. It is then taken out after washing with water. The purified mercury, so obtained is preferable for preparation of Parpam, Chenduram, Kuru,and Kuligai.

- Gunapadam thathu Jeeva Vaguppu, Pg.no: 244

Gandhagam

Gandhagam is heated with Ponankanni (Alternanthera sessilis) juice for 3 hours by using mild flame. Then the Gandhagam is washed with water and taken.

- Agathiyar attavanai vaagadam, Pg. no: 23

Lingam

Lime juice, cow's milk and Indian Acalypha juice are mixed in equal proportion and allowed to fuse Cinnabar so as to get it in a consolidated potency state.

- Gunapadam Thathu Jeeva Vagupu, Pg.no: 272

Thalagam

Thalagam is bundled and boiled with lime stone water by Thulayanthiram method with dipping.

- Saraku suthi sei muraigal, pg.no:67

Manosilai

Manosilai is taken and grinded with lemon juice for 3 hours in a kalvam.

- Sikicha rathina deepam, Pg.no:37

Vellai Paadanam

Vellai Paadana powdered and loosely bundled with a thick cloth. Milagu (Piper nigrum) is triturated with and dissolved in the one litre juice of Sirukeerai (Amaranthus tricolor) in a mud pot.By the method of thulayanthiram, bundled paadanam is dipped into the mixture without touching the vessel. It is boiled using low intensity fire till drying of the mixture and washed with water and taken out .Repeat the process for 3 times.

- Gunapadam Thathu Jeeva Vagupu, Pg.no: 359

Pooram

Vetrilai(Piper betel) and Milagu(Piper nigrum) is taken separately equal to the weight of Pooram. Grinded paste of Vetrilai and Milagu is mixed with water in a mud pot. By the method of thulaiyandram prepared pooram is tied immersed into the mixture and taken after boiled well.

- Sikicha rathina deepam, Pg.no:37

Gaantham

Gaantham is taken and ground with lemon juice for 3 hours and soaked with lime juice for 45 minutes to get it in a purified form.

-Saraku suthi sei muraigal, pg.no:28

METHOD OF PREPARATION:

Ingredients:

Purified *Rasam* (Mercury)	-1 *palam*(35 gm),
Purified *Gandhagam* (Sulphur)	-1 *palam* (35 gm),
Purified *Lingam* (Cinnabar)	-1 *palam* (35 gm),
Purified *Thalagam* (Arsenic trisulphide)	-1 *palam* (35 gm),
Purified *Manosilai* (Arsenic disulphide)	-1 *palam* (35 gm),
Purified *Vellai Paadanam* (White arsenic)	-1 *palam* (35 gm),
Purified *Pooram* (Calomel)	-1 *palam* (35 gm),
Purified *Gaantham* (Magnetic oxide of iron)	-1 *palam* (35 gm),
Purified *Saathikkai* (Myristica fragrens	-1 *palam* (35 gm)
Juice of *Kuppaimaeni* (Acalypha indica)	-*Required quantity.*
Purified *Soatruppu* (Common salt)	-2 padi

Purified Rasam and Gandhagam are ground well till it becomes dark colour. Except salt, all other ingredients are powdered separately and ground with Kuppaimaeni juice for one hour. The above paste is applied on a soft cloth and dried. The total amount of salt is divided into two; one half is put at the bottom of a mud pot and the processed cloth is placed above the salt. Then the remaining amount of salt is put over the processed cloth. The above set is covered with a suitable mud plate. The margins are covered with 7 layers of clay cloth, dried and is burnt for 3 saamam (9 hr). Being cooled, the lid is opened and the sublimated medicine over the wall of the vessel is kept in a container.

Therapeutic dose: 1/4 – 1/2 *kundri* (32.5 – 65mg), twice a day or once in morning according to the temperament of body.

Adjuvant **:** 1/2 Aalaku (168 ml) cow's milk

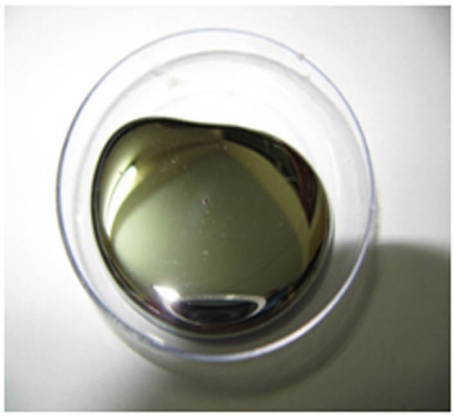
Therapeutic uses : Puttru(Cancer in cheek- breast mouth sexual organs, legs), Vellai Kuttam (vitiligo), Kandamaalai (cervical adenitis).

Precautions **:** Avoid tamarind. Fried salt should be used..

- Anuboga vaithiya navaneetham, part - 10, page no 53

year of edition -1995

RASAM



Unpurified

Purified

GANDHAGAM

Unpurified

Purified

LINGAM

Unpurified

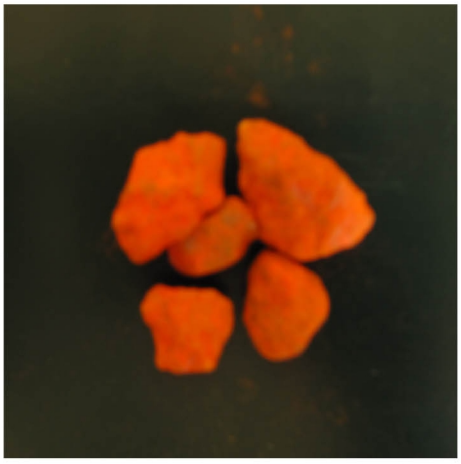
Purified

THALAGAM

Unpurified

Purified

MANOSILAI



Unpurified

Purified

VELLAI PAADANAM

Unpurified

Purified

POORAM

Unpurified

Purified

KAANTHAM

Unpurified

Purified

SAATHIKKAI

KUPPAIMAENI

PUTTRU PATHANGAM

4.2. QUALITATIVE ANALYSIS

PHYSICO-CHEMICAL PROPERTIES

EXPERIMENTAL PROCEDURES: Done at Biochemistry Lab, NIS and SAIF, IIT Chennai-36.

Colour:

About 50 gm of **Puttru Pathangam** was taken in a clean glass beaker and tested for its colour by viewing against a white opaque back ground under direct sunlight.

Odour:

About 50 gm of the **Puttru Pathangam** was placed in 100 ml of beaker and tested for its odour by wafting the air above the beaker.

pH

The ph of the **Puttru Pathangam** was estimated as per the method prescribed in the Indian standard (IS) - 6940(1982). One gram of **Puttru Pathangam** was taken into a 100ml graduated cylinder containing about 50 ml of water and filled up to the mark with water. The cylinder was stopped and shaken vigorously for two minutes and the suspension was allowed to settle for an hour at 25 to 27˚C. About 25 ml of the clear aqueous solution was transferred into a 50 ml beaker and tested for pH using DIGISUN digital PH meter (DIGISUN electronics, Hyderabad, India).

Determination of Ash value:

Two gms of the **Puttru Pathangam** was weighed accurately in tarred platinum or silica dish and incinerated at a temperature not exceeding 450˚C until free from carbon, then cooled and weighed. Calculate the percentage of ash with reference of the air dried drug.

Water Soluble Ash:

To the Gooch crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered glass crucible or on ash less filter paper. Washed with hot water and ignited in a crucible for 15 minutes at a

temperature not exceeding 450˚C. Subtract the weight of the insoluble matter from the weight of the ash. The difference of the weight represents the water soluble ash. Calculate the percentage of water soluble ash with reference to the air dried drug.

Acid Soluble Ash:

Ash was boiled for 5 minutes with 25 ml of 1:1 diluted Hcl. The insoluble matter was collected in a Gooch crucible and placed on an ash less filter paper, washed with water and then ignited. Finally cooled in a desiccator and weighed. The percentage of insoluble ash was calculated with reference to the air dried drug.

Loss on drying:

Five grams of the **Puttru Pathangam** was heated in a hot oven at 105˚c to a constant weight. The percentage loss of weight was calculated as per procedure.

PHYSICO CHEMICAL PROPERTIES

EXPERIMENT	OBSERVATION	INFERENCE
Appearance of the sample		
Solubility: **a.** A little of the sample is shaken well with distilled water. **b.** A little of the sample is shaken well with con. Hcl and Con. H2SO4.	Sparingly soluble Insoluble	Presence of Silicate Absence of Silicate
Action of Heat: A small amount of the sample is taken in a dry test tube and heated gently first and then strong.	white fumes evolved No white fumes evolved.	Presence of Carbonate Absence of Carbonate
Flame Test: A small amount of the sample is made into a paste with Con.Hcl in a watch glass and introduced into non-luminous part of the Bunsen flame.	Appearance of bluish green flame No appearance of bluish green flame	Presence of Copper Absence of Copper
Ash Test: A filter paper is soaked into a mixture of sample and cobalt nitrate solution and introduced into the Bunsen flame and ignited.	Appearance of yellow colour flame No appearance of yellow colour flame	Presence of Sodium Absence of Sodium

Preparation of extract:

5g of sample was taken in a 250 ml of clean beaker and 50 ml of distilled water was added to it. Then it was boiled well for about 10 min. Then it is allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation is used for the qualitative analysis of acidic and basic radicals.

TEST FOR BASIC RADICALS

PROCEDURE	OBSERVATION	INFERENCE
Test for Potassium: A pinch of sample is treated with 2ml of sodium nitrate solution and then treated with 2ml of cobalt nitrate in 30% of glacial acetic acid	Formation of Yellow colour precipitate No Formation of Yellow colour precipitate	Presence of Potassium Absence of Potassium
Test for Calcium: 2 ml of extract is taken in a clean test tube. To this add 2 ml of 4% ammonium oxalate solution.	Formation of White colour precipitate No formation of White colour precipitate	Presence of Calcium Absence of Calcium
Test For Magnesium: To 2ml of extract, sodium hydroxide solution is added in drops to excess	Formation of White colour precipitate No formation of White colour precipitate	Presence of Magnesium Absence of Magnesium
Test For Ammonium: To 2ml of extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added.	Appearance of Brown colour No appearance of Brown colour	Presence of Ammonium Absence of Ammonium
Test For Sodium: 2 pinches of the substance is made into paste by using Hcl and introduced into the blue flame of Bunsen burner.	Appearance of intense Yellow colour No appearance of intense Yellow colour	Presence of Sodium Absence of Sodium
Test for Iron (Ferrous): The extract is treated with Conc. HNO_3 and ammonium thiocynate.	Appearance of Blood red colour. No appearance of Blood red colour	Presence of Ferrous iron Absence of Ferrous iron
Test For Zinc: To 2m1 of the extract sodium	Formation of White colour precipitate	Presence of Zinc

hydroxide solution is added in drops to excess.	No formation of White colour precipitate	Absence of Zinc
Test For Aluminium: To the 2m1of the extract sodium hydroxide is added in drops to excess.	Characteristic changes No Characteristic changes	Presence of Aluminium Absence of Aluminium
Test For Lead: 2 ml of extract is added with 2ml of potassium iodide solution.	Formation of yellow colour precipitate No formation of yellow colour precipitate	Presence of Lead Absence of Lead
Test for Copper: a. One pinch of substance is made into paste with con. Hcl in a watch glass and introduced into the non-luminous part of the flame. b. 2 ml of extract is added with excess of ammonia solution.	Formation of Blue colour Precipitate. No formation of Blue colour Precipitate.	Presence of Copper Absence of Copper
Test For Mercury: 2m1 of the extract is treated with 2ml of sodium hydroxide solution.	Formation of Yellow precipitate No formation of Yellow precipitate	Presence of Mercury Absence of Mercury
Test for Arsenic: 2m1 of the extract is treated With 2ml of sodium hydroxide solution.	Formation of Brownish red precipitate No formation of Brownish red precipitate	Presence of Arsenic Absence of Arsenic

TEST FOR ACID RADICALS

PROCEDURE	OBSERVATION	INFERENCE
Test for Sulphate: 2 ml of the extract is added to 5 % barium chloride solution.	Formation of white precipitate No formation of white precipitate	Presence of Sulphate Absence of Sulphate
Test for Chloride : The extract is treated with Silver nitrate solution.	Formation of White precipitate No formation of White precipitate	Presence of Chloride Absence of Chloride
Test for Phosphate : The extract is treated with ammonium molybdate and 2ml of conc. HNO_3.	Formation of Yellow precipitate No formation of Yellow precipitate	Presence of Phosphate Absence of Phosphate
Test for Carbonate : The substance is treated with Conc. Hcl.	Formation of effervesence No formation of effervesence	Presence of carbonate Absence of carbonate
Test for fluoride &oxalate: 2ml of extract is added with 2ml of dil. acetic acid and 2ml calcium chloride solution and heated.	Formation of cloudy appearance No formation of cloudy appearance	Presence of Fluoride & Oxalate Absence of Fluoride & Oxalate
Test For Nitrate: 1gm of the substance is heated with copper turnings and concentrated H2SO4 and viewed the test tube vertically down	Characteristic changes No characteristic changes	Presence of Nitrate Absence of Nitrate

OTHER CONSTITUENTS

PROCEDURE	OBSERVATION	INFERENCE
Test for Starch: The extract is added with weak iodine solution.	Formation of blue colour No formation of bluea colour	Presence of Starch Absence of Starch
Test for Reducing Sugar: 5 ml of Benedict's qualitative solution is taken in a test tube and allowed to boil for 2 min. Add 8 to 10 drops of extract and again boil it for 2min. The colour changes are noted.	Brickred colour No brickred colour	Presence of Reducing Sugar Absence of Reducing Sugar
Test for Alkaloids: **a**. 2ml of the extract is treated with 2ml of potassium Iodide solution **b.** 2ml of extract is treated with 2ml of picric acid **c.** 2ml of the extract is treated with 2ml of phosphotungstic acid	No Red colour Appearance of Yellow colour No white precipitate	- Presence of Alkaloids -
Test for amino acids: Dilute extract + 2ml of Ninhydrin's solution.	Appearance of violet colour No appearance of violet colour	Presence of Amino acids Absence of Amino acids
Test for Tannic acid : 2ml extract is treated with	Formation of Blue black precipitate	Presence of Tannic acid

Ferric chloride.	No formation of Blue black precipitate	Absence of Tannic acid
Test for unsaturated Compound: 2ml of the extract is treated with 2 ml of potassium permanganate solution is added.	Appearance of Green colour Appearance of Red colour Appearance of Violet colour Appearance of Blue colour	Presence of Oxyquinole, Epinephrine and Pyro catechol Presence of Anti pyrine, Aliphatic amino acids and Meconic acid Presence of Apomorphine Salicylate and Resorcinol Presence of Morphine, Phenol cresol and Hydroquinone

4.3 QUANTITATIVE ANALYSIS

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES)

Introduction

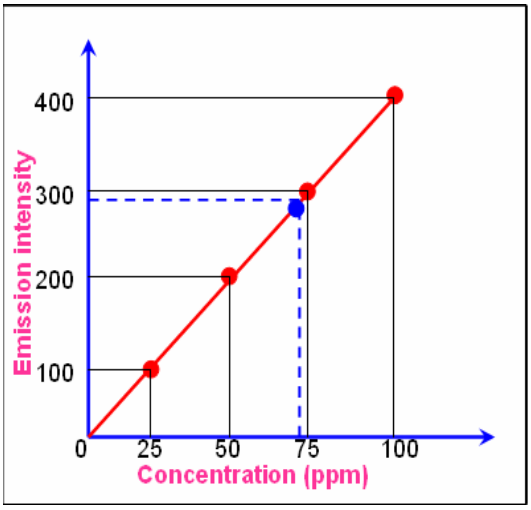
The elemental composition of a sample is often an important part of the information needed to assess its properties. Hence there is a need for sensitive scientific instrumentation like ICP-OES which plays a pivotal role in the determination of these elements. ICP-OES is widely employed for the estimation of metals and metalloids at trace, minor and major concentrations.

Principle

In this technique, the high temperature plasma source atomizes the sample and excites the atoms resulting in emission of photons .The atoms of each element in the sample emit specific wavelength of light. The emission spectrum from the plasma is dispersed by an optical spectrometer, so that intensities of the individual wavelength can be measured. The number of photons emitted is directly proportional to the concentration of the element. The photon may be detected either sequentially or simultaneously. Quantitative analysis is achieved by measuring the intensity of these specific wavelengths and after performing the calibration using known standards.

Extraction of information

Obtaining qualitative information, i.e., what elements are present in the sample, involves identifying the presence of emission at the wavelengths characteristic of the elements of interest. Obtaining quantitative information, i.e., how much of an element is in the sample, can be accomplished using plots of emission intensity versus concentration called calibration curves. Typical calibration graph is illustrated below.



Typical ICP Calibration curve

Perkin Elmer Optima 5300DV

40 M Hz RF generator;

Range: 165-782 nm;

Experimental Procedure: Done at SAIF, IIT Madras, Chennai-36

Sample preparation – Microwave Digestion

Weigh 0.25g of test sample and transfer into a liner provided with the instrument. Slowly add 9ml of Nitric acid or Sulphuric acid such that no piece of sample sticks on the slides. Mix thoroughly and allow reacting for few minutes. Place the liner in the vessel jacket. Close the screw cap hand-tight in clockwise direction. Seal the vessel and place in the rotor fixed in microwave. Set temperature to 180°C for 5 minutes; hold at 180°C for least 10 minutes. Allow the vessels to cool down to a vessel interior temperature below 60°C and to a vessel surface temperature (IR) below 50°C before removing the rotor. The digested sample was made upto 100ml with millipore water. If visible insoluble particles exist, solution could be filtered through whatmann filter paper. Transfer the digested solution into plastic containers and label them properly.

X-RAY FLUORESCENCE (XRF)

X-ray fluorescence (XRF) spectrometer is an x-ray instrument used for routine, relatively non-destructive chemical analyses of rocks, minerals, sediments and fluids. It works on wavelength-dispersive spectroscopic principles that are similar to an electron microprobe (EPMA). However, an XRF cannot generally make analyses at the small spot sizes typical of EPMA work (2-5 microns), so it is typically used for bulk analyses of larger fractions of geological materials. The relative ease and low cost of sample preparation, and the stability and ease of use of x-ray spectrometers make this one of the most widely used methods for analysis of major and trace elements in rocks, minerals, and sediment.

Fundamental Principles of X-Ray Fluorescence (XRF)

The XRF method depends on fundamental principles that are common to several other instrumental methods involving interactions between electron beams and x-rays with samples, including: X-ray spectroscopy (e.g., SEM - EDS), X-ray diffraction (XRD), and wavelength dispersive spectroscopy. Analysis of major and trace elements in geological materials by x-ray fluorescence is made possible by the behavior of atoms when they interact with radiation. When materials are excited with high-energy, short wavelength radiation (e.g., X-rays), they can become ionized. If the energy of the radiation is sufficient to dislodge a tightly-held inner electron, the atom becomes unstable and an outer electron replaces the missing inner electron. When this happens, energy is released due to the decreased binding energy of the inner electron orbital compared with an outer one. The emitted radiation is of lower energy than the primary incident X-rays and is termed fluorescent radiation. Because the energy of the emitted photon is characteristic of a transition between specific electron orbital in a particular element, the resulting fluorescent X-rays can be used to detect the abundances of elements that are present in the sample.

Procedure

The analysis of major and trace elements in geological materials by XRF is made possible by the behavior of atoms when they interact with X-radiation. An XRF spectrometer works because if a sample is illuminated by an intense X-ray beam, known

as the incident beam, some of the energy is scattered, but some is also absorbed within the sample in a manner that depends on its chemistry. The incident X-ray beam is typically produced from a Rh target, although W, Mo, Cr and others can also be used, depending on the application.

When this primary X-ray beam illuminates the sample, it is said to be excited. The excited sample in turn emits X-rays along a spectrum of wavelengths characteristic of the types of atoms present in the sample. How does this happen? The atoms in the sample absorb X-ray energy by ionizing, ejecting electrons from the lower (usually K and L) energy levels. The ejected electrons are replaced by electrons from an outer, higher energy orbital. When this happens, energy is released due to the decreased binding energy of the inner electron orbital compared with an outer one. This energy release is in the form of emission of characteristic X-rays indicating the type of atom present. If a sample has many elements present, as is typical for most minerals and rocks, the use of a Wavelength Dispersive Spectrometer much like that in an EPMA allows the separation of a complex emitted X-ray spectrum into characteristic wavelengths for each element present. Various types of detectors (gas flow proportional and scintillation) are used to measure the intensity of the emitted beam. The flow counter is commonly utilized for measuring long wavelength (>0.15 nm) X-rays that are typical of K spectra from elements lighter than Zn. The scintillation detector is commonly used to analyze shorter wavelengths in the X-ray spectrum (K spectra of element from Nb to I; L spectra of Th and U). X-rays of intermediate wavelength (K spectra produced from Zn to Zr and L spectra from Ba and the rare earth elements) are generally measured by using both detectors in tandem. The intensity of the energy measured by these detectors is proportional to the abundance of the element in the sample. The exact value of this

proportionality for each element is derived by comparison to mineral or rock standards whose composition is known from prior analyses by other techniques. X-Ray fluorescence is particularly well-suited for investigations that involve

- bulk chemical analyses of major elements (Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K, P) in rock and sediment

- bulk chemical analyses of trace elements (in abundances >1 ppm; Ba, Ce, Co, Cr, Cu, Ga, La, Nb, Ni, Rb, Sc, Sr, Rh, U, V, Y, Zr, Zn) in rock and sediment - detection limits for trace elements are typically on the order of a few parts per million

X-ray fluorescence is limited to analysis of

- relatively large samples, typically > 1 gram
- materials that can be prepared in powder form and effectively homogenized
- materials for which compositionally similar, well-characterized standards are available
- materials containing high abundances of elements for which absorption and fluorescence effects are reasonably well understood.

In most cases for rocks, ores, sediments and minerals, the sample is ground to a fine powder. At this point it may be analyzed directly, especially in the case of trace element analyses. However, the very wide range in abundances of different elements, especially iron, and the wide range of sizes of grains in a powdered sample, makes the proportionality comparison to the standards particularly troublesome. For this reason, it is common practice to mix the powdered sample with a chemical flux and use a furnace or gas burner to melt the powdered sample. Melting creates a homogenous glass that can be analyzed and the abundances of the (now somewhat diluted) elements calculated.

Strengths

X-Ray fluorescence is particularly well-suited for investigations that involve:

- bulk chemical analyses of major elements (Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K, P) in rock and sediment
- bulk chemical analyses of trace elements (>1 ppm; Ba, Ce, Co, Cr, Cu, Ga, La, Nb, Ni, Rb, Sc, Sr, Rh, U, V, Y, Zr, Zn) in rock and sediment.

Experimental Procedure: Done at Sastra University,Tanjore.

FOURIER TRANSFORM - INFRA RED SPECTROSCOPY

Analyser: PERKIN ELMER – SPECTRUM ONE

Introduction

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure. The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy.

In IR spectroscopy, the resonance absorption is made possible by the change in dipole moment accompanying the vibrational transition. The Infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible.

The IR spectroscopy is carried out by using Fourier transform technique.

Principle

Infra red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction, electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecular and electronic structure of the substance and with intra- and inter molecular interactions.

FT-IR spectroscopy is used primarily for qualitative and quantitative analysis of organic compounds, and also for determining the chemical structure of inorganic materials. The region between 500-4000 wave numbers is referred to as the finger print region. Absorption bands in this region are generally due to **intra molecular** phenomena and are highly specific for each material. The specificity if these bands allow computerized data searches to be performed against reference libraries to identify a material.

Table of Characteristic IR Absorptions

frequency, cm–1	bond	functional group
3640–3610 (s,sh)	O–H stretch, free hydroxyl	alcohols, phenols
3500–3200 (s,b)	O–H stretch, H–bonded	alcohols, phenols
3400–3250 (m)	N–H stretch	1°, 2° amines, amides
3300–2500 (m)	O–H stretch	carboxylic acids
3330–3270 (n, s)	–C≡C–H: C–H stretch	alkynes (terminal)
3100–3000 (s)	C–H stretch	aromatics
3100–3000 (m)	=C–H stretch	alkenes
3000–2850 (m)	C–H stretch	alkanes
2830–2695 (m)	H–C=O: C–H stretch	aldehydes
2260–2210 (v)	C≡N stretch	nitriles
2260–2100 (w)	–C≡C– stretch	alkynes
1760–1665 (s)	C=O stretch	carbonyls (general)
1760–1690 (s)	C=O stretch	carboxylic acids
1750–1735 (s)	C=O stretch	esters, saturated aliphatic

1740–1720 (s)	C=O stretch	aldehydes, saturated aliphatic
1730–1715 (s)	C=O stretch	α,β–unsaturated esters
1715 (s) C=O	stretch	ketones, saturated aliphatic
1710–1665 (s)	C=O stretch	α,β–unsaturated aldehydes, ketones
1680–1640 (m)	–C=C– stretch	alkenes
1650–1580 (m)	N–H bend	1° amines
1600–1585 (m)	C–C stretch (in–ring)	aromatics
1550–1475 (s)	N–O asymmetric stretch	nitro compounds
1500–1400 (m)	C–C stretch (in–ring)	aromatics
1470–1450 (m)	C–H bend	alkanes
1370–1350 (m)	C–H rock	alkanes
1360–1290 (m)	N–O symmetric stretch	nitro compounds
1335–1250 (s)	C–N stretch	aromatic amines
1320–1000 (s)	C–O stretch	alcohols, carboxylic acids, esters, ethers
1300–1150 (m)	C–H wag (–CH2X)	alkyl halides
1250–1020 (m)	C–N stretch	aliphatic amines
1000–650 (s)	=C–H bend	alkenes
950–910 (m)	O–H bend	carboxylic acids
910–665 (s, b)	N–H wag	1°, 2° amines
900–675 (s)	C–H “oop”	aromatics
850–550 (m)	C–Cl stretch	alkyl halides
725–720 (m)	C–H rock	alkanes
700–610 (b, s)	–C≡C–H: C–H bend	alkynes
690–515 (m)	C–Br stretch	alkyl halides

m=medium, w=weak, s=strong, n=narrow, b=broad, sh=sharp

Sampling techniques:

There are a variety of techniques for sample preparation depending on the physical form of the sample to be analyzed. Solid : KBr or Nujol mull method. Liquid : CsI / TlBr Cells Gas : Gas cells

Experimental Procedure: Done at SAIF, IIT Madras, Chennai-36.

KBr Method

- The sample was grounded using- an agate mortar and pestle to give a very fine powder.
- The finely powder sample was mixed with about 100mg dried KBr salt.
- The mixture was then pressed under hydraulic press using a die to yield a transparent disc (measure about 13mm diameter and 0.3mm in thickness), through which the beam of spectrometer passed.

SCANNED ELECTRON MICROSCOPY (SEM)

A SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross-sections. The primary electron beam interacts with the sample in a number of key ways:-

- ❖ Primary electrons generate low energy secondary electrons, which tend to emphasize the topographic nature of the specimen.
- ❖ Primary electrons can be backscattered which produces images with a high degree of atomic number (Z) contrast.
- ❖ Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection. The X-rays emitted are characteristic of the elements in the top few μm of the sample.

The SEM is carried out by using FEI-Quanta FEG 200-High Resolution Instrument.

Resolution : 1.2 nm gold particle separation on a carbon substrate

Magnification : From a min of 12x to greater than 1, 00,000 X

Application : To evaluate grain size, particle size distributions, material homogeneity and inter metallic distributions.

Experimental Procedure: Done at SAIF, IIT Madras, Chennai-36

Sample preparation:

Sample preparation can be minimal or elaborate for SEM analysis, depending on the nature of the samples and the data required. Minimal preparation includes acquisition of a sample that will fit into the SEM chamber and some accommodation to prevent charge build-up on electrically insulating samples. Most electrically insulating samples are coated with a thin layer of conducting material, commonly carbon, gold, or some other metal or alloy. The choice of material for conductive coatings depends on the data to be acquired: carbon is most desirable if elemental analysis is a priority, while metal coatings are most effective for high resolution electron imaging applications. Alternatively, an electrically insulating sample can be examined without a conductive coating in an instrument capable of "low vacuum" operation.

4.4 TOXICOLOGICAL EVALUATION OF

PUTTRU PATHANGAM

Scope of the work:

Preclinical drug development is a stage that begins before clinical trials during which important safety and pharmacology data are collected. Regulatory toxicity studies are conducted in animals to identify possible hazards from which an assessment of risk to humans is made by extrapolation. The choice of animal species is based on the similarities of its metabolism to human.

The goals of the non clinical safety evaluation includes

- Categorization of toxic effects with respect to target organs, dose dependence, relationship to exposure and potential reversibility. This information is important for the estimation of an initial safe starting dose for the human trial.
- The identification of specific parameters for clinical monitoring for potential adverse effect.

Systemic toxicity studies

Acute oral dose toxicity study

Single dose studies (acute oral dose toxicity) in animals are essential for any pharmaceutical products intended for human use .the information obtained from these studies is useful in choosing doses for repeated dose studies, providing preliminary identification of target organs of toxicity and occasionally revealing delayed toxicity. Acute toxicity studies may also aid in the selection of starting doses for phase I human studies and provide information relevant to acute over dosing in humans

Repeated dose systemic toxicity studies

The primary goal of repeated dose toxicity studies is to characterize the toxicological profile of the cell compound following repeated administration. This includes identification of potential target organs of toxicity and exposure /response

relationship and may include the potential reversibility of toxic effects. This information should be part of the safety assessment to support the conduct of human clinical trials and the approval of marketing authorization.

PLAN OF WORK

The following studies carried out in **PPM** are

Acute oral toxicity study – OECD 423 guideline

Repeated oral toxicity study – OECD 407 guideline

The toxicity studies were evaluated after getting permission from the Institutional Animal Ethical Committee clearance**. (1248/ac/09/CPCSEA/04/IAEC 2011).**

Test drug : **PUTTRU PATHANGAM - (PPP)**

Study Place : Animal house, NIS.

PRECLINICAL ANIMAL TOXICITY STUDIES OF IN PUTTRU PATHANGAM RODENTS

ACUTE ORAL TOXICITY – OECD-423

Introduction:

1. The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods. The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.
2. In principle, the method is not intended to allow the calculation of a precise LD_{50}, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test. The method allows for the determination of an LD_{50} value only when at

least two doses result in mortality higher than 0% and lower than 100%. The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

PRINCIPLE

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per, step sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.;

- no further testing is needed,
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

METHODOLOGY

1. Selection of animal species

The preferred rodent species is the rat, although other rodent species may be used. Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval within ±20 % of the mean weight of any previously dosed animals. The Wistar albino female species were selected.

2. Housing and feeding conditions:

The temperature in the experimental animal room should be 22 ± 3ºC. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hour light, 12 hour dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Administration of doses:

PPP suspended in 10% aqueous tween 80 solution with uniform mixing and was administered to the groups of wistar rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted for 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three animals are used for each step. The dose level of 5, 50, 300 and 2000 mg/kg body weight was administered stepwise. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 hour prior to the administration of the test suspension. Finally, the number of survivors were noted after 24 hour and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

3. Test substance and Vehicle:

The **PPP** is freely soluble in water and the suspension was made with tween80 solution for dose accuracy and easy administration in animals.

4. Justification for choice of vehicle:

The vehicle selected as per the standard guideline which is pharmacologically inert and easy to employ for new drug development and evaluation technique.

5. Test animals and Test conditions:

Sexually mature Wistar albino rats (110-128g) were obtained from Sri Raghavendra Enterprises, Bangalore and King Institute Chennai. All the animals were kept under standard environmental condition (22 ± 3ºC). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore). Rats were deprived of food but not water (16-18 h) prior to administration of the PPP. The principles of laboratory animal care were followed.

OBSERVATIONS:

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal. Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern, sleep and coma. The principles and criteria summarised in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed. When animals are killed for humane reasons or found dead, the time of death should was recorded.

Body weight:

Individual weight of animals was determined before the test substance was administered and at least weekly thereafter. Weight changes was calculated and recorded. At the end of the study, surviving animals were weighed and humanely killed.

RESULTS

All data were summarised in tabular form, (Table-7) showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility, and necropsy findings.

28-DAYS REPEATED DOSE ORAL TOXICITY STUDY OF PUTTRU PATHANGAM IN RATS

Test Substance	**: PPP**
Animal Source	: King Institute,Chennai
Animals	: Male and Female wistar albino Rats
Study place	: Animal house, NIS
Age	**:** 6-8 weeks
Body Weight on Day 0	**:** Males: Mean 108.86g Females: Mean 112.68g
Acclimatization	**:** Seven days prior to dosing.
Veterinary examination	**:** Prior to and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking on fur with picric acid.
Diet	**:** Pelleted feed supplied by Sai meera foods Pvt Ltd, Bangalore.
Water	**:** Aqua guard portable water in polypropylene bottles ad libitum.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** Between 22°C ±3ºC,
Relative humidity	**:** Between 30% and 70%,
Air changes	**:** 10 to 15 per hour
Dark and light cycle	**:** 12: 12 hours.

Justification for dose selection:

As stated in results of acute oral toxicity studies in wistar rats indicated that LD cutoff value of PPP was 200 mg/ kg body weight. Mortality was observed in 2 animals of 300mg/kg body weight. On the basis of these results, the doses selected for the study were X, 5X and 10X. The X value was calculated with the human therapeutic dose and the body surface area of the rat. The oral route was selected since oral route is considered to be a proposed therapeutic route.

Preparation and administration of dose:

PPP was suspended on 10% aqueous tween 80 and administered to animals at the dose levels of X, 5X and 10X. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

Methodology:

Randomization, Numbering and Grouping of animals.

Six rats (3 male + 3 female) in each group randomly divided in to 4 groups for treating the test drug up to 28 days.

Group I : Control receiving vehicle (10% aqueous tween 80 solution).

Group II : PPP at the dose level of X dose (1.17mg).

Group III : PPP at the dose level of 5X dose (5.85mg).

Group IV : PPP at the dose level of 10X dose (11.7mg).

The study will be carried out as per OECD Guideline 407 (28-Days Repeated Oral Dose Toxicity in Rodents). The animals will be divided in four groups each group consist of 6 animals (3 males and 3 females). One group will serve as control and the other three groups for test drug at three dose levels (low, mid and high) for 28 days.

Observations:

During the study, body weight of the animals will be evaluated weekly; water, food consumption and mortality events will be evaluated daily. By the end of 28 days, the animal will be sacrificed by excessive anesthesia. Blood will be collected in all overnight (12 hours) fasted rats through jugular vein and it will be processed for below mentioned investigations. Vital organs will be collected from the animals and subjected to histopathology.

LABORATORY TEST:

Complete haemogram

Renal function test

Liver function test

GROSS NECROPSY:

It includes examination of the external surface of the body, all orifices, and organs like brain, lungs, heart, spleen, liver, kidneys, adrenals and sex organs of all animals.

HISTOPATHOLOGY:

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low dose treated animals, then the mid dose treated animals will also be examined. Liver, kidney, lungs, spleen, heart and stomach will be collected from all animals and preserved in 10% buffered neutral formalin, sliced 5 or 6µm sections and it will be stained with hematoxylin and eosin, examined for histopathological changes.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, haematology and blood chemistry were subjected to One-way ANOVA followed by dunnet‘t‘ test using a computer software programme -INSTAT-V3 version.

PHYSICO CHEMICAL ANALYSIS

Colour characters of Puttru Pathangam

Table.3.

S No	Nature of the drug	Under ordinary light	Under ultra violet light
1.	Powdered material	Brown	Brown

Physico chemical properties of Puttru Pathangam

S No.	Parameters	Values obtained (%w/w)
1.	Total ash value	8.45
2.	Acid insoluble ash	0.75
3.	Water soluble ash	7.14
4.	Moisture content	9.22

Heavy/toxic metals in Puttru Pathangam

Lead	BDL
Cadmium	BDL
Mercury	3.842mg/L
Arsenic	BDL

Colour, nature and percent yields of extracts of **Puttru Pathangam**

S.No.	Extract Solvents	Colour	Nature	%Yield(w/w)	pH
1.	Water	Brown	Solid	48	3.2-3.5

QUALITATIVE ANALYSIS

CHEMICAL ANALYSIS

The qualitative analysis of **Puttru Pathangam** shows the presence of

- Iron
- Chloride
- Phosphate
- Unsaturated compound

QUANTITATIVE ANALYSIS

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY

Table. 4.

S.no.	Elements	Wavelength in nm	PuttruPathangam (ppm)
1.	Arsenic	As193.696	BDL*
2.	Calcium	Ca 317.933	15.856
3.	Cadmium	Cd 226.502	BDL*
4.	Mercury	Hg 253.652	3.187
5.	Iron	Fe 238.204	1.356
6.	Potassium	K 766.490	45.426
7.	Sodium	Na 589.592	17.952
8.	Phosphorus	P 213.617	9.784
9.	Lead	Pb 230.204	BDL*

* BDL = below detection limit, ppm – Parts per million

X-RAY FLUORESCENCE (XRF)

Table.5

Oxide form			Elemental form		
Formula	**Z**	**Concentration**	**Formula**	**Z**	**Concentration**
Hg	80	42.14%	**Hg**	80	42.14%
As2O3	33	8.48%	**As**	33	8.14%
SO3	16	6.51%	**O**	8	16.15%
Cl	17	5.60%	**Cl**	17	5.60%
MgO	12	5.59%	**Mg**	12	3.37%
SiO2	14	0.57%	**S**	16	2.61%
Al2O3	13	0.44%	**Si**	14	0.27%
Fe2O3	26	0.20%	**Al**	13	0.23%
SeO2	34	0.14%	**Fe**	26	0.14%
TiO2	22	0.12%	**Se**	34	0.10%
PbO	82	0.09%	**Pb**	82	0.08%
MnO	25	0.09%	**Ti**	22	0.07%
Tl	81	0.02%	**Mn**	25	0.07%
NiO	28	0.01%	**Tl**	81	0.02%
			Ni	28	90 PPM

FOURIER TRANSFORM - INFRA RED SPECTROSCOPY

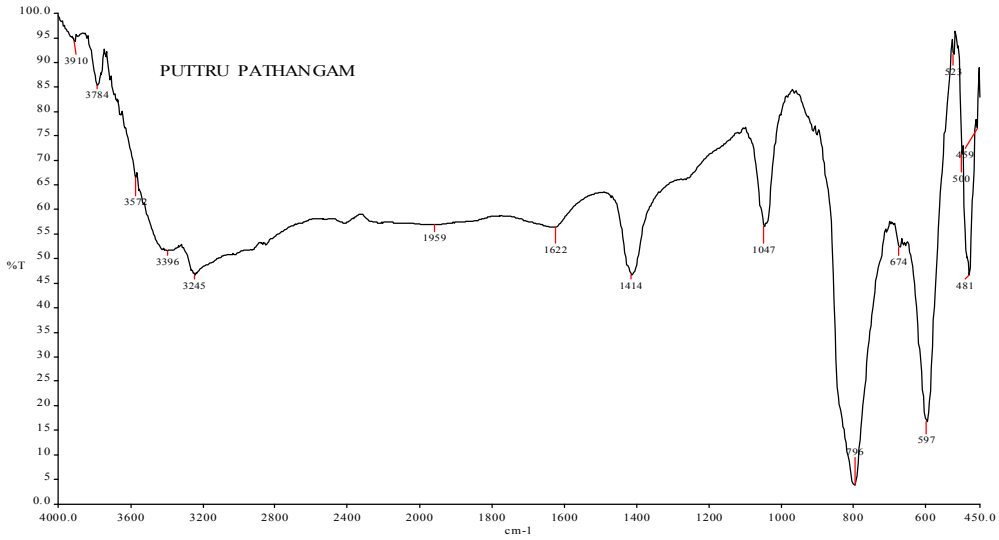


Table of Characteristic IR Absorptions

Table.6

frequency, cm–1	bond	functional group
3640–3610 (s, sh)	O–H stretch, free hydroxyl	alcohols, phenols
3500–3200 (s,b)	O–H stretch, H–bonded	alcohols, phenols
3300–2500 (m)	O–H stretch	carboxylic acids
1650–1580 (m)	N–H bend	1° amines
1250–1020 (m)	C–N stretch	aliphatic amines
910–665 (s, b)	N–H wag	1°, 2° amines
900–675 (s)	C–H “oop”	aromatics
850–550 (m)	C–Cl stretch	alkyl halides
700–610 (b, s)	–C≡C–H: C–H bend	alkynes
690–515 (m)	C–Br stretch	alkyl halides

m=medium, w=weak, s=strong, n=narrow, b=broad, sh=sharp

SCANNED ELECTRON MICROSCOPY (SEM)

Determination of particle size of Puttru Pathangam

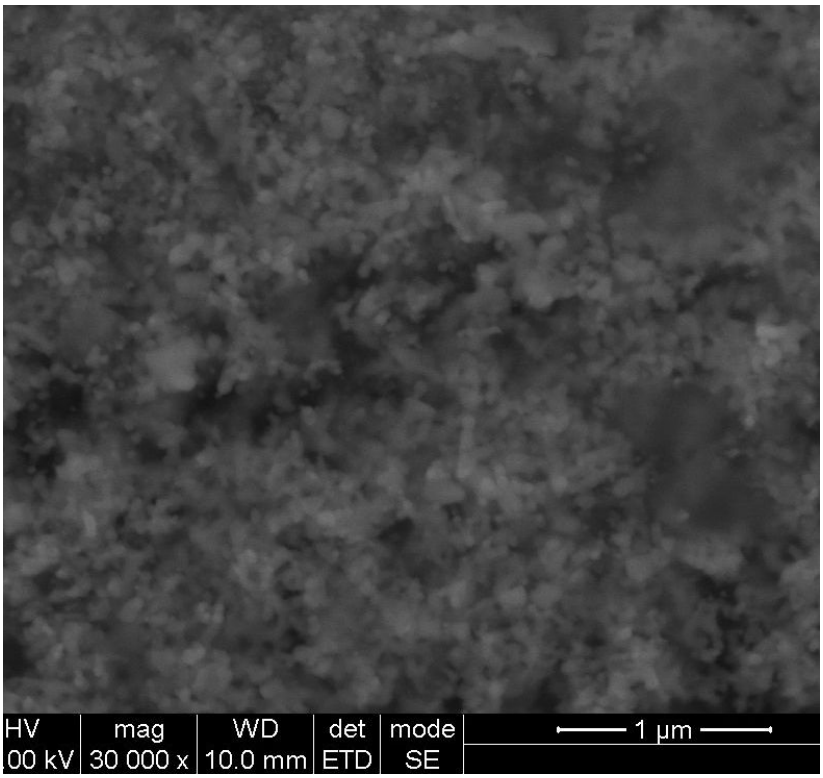


Fig.4. HR SEM – PUTTRU PATHANGAM

1. Particle size is 0.25 to 0.5 µ (micron).

2. Cumulative distribution of the particles with bulk density is seen.

3. Surface is smooth in nature.

TOXICITY STUDY

ACUTE ORAL TOXICITY STUDY

All the data´s were summarized in the form of table (5) showing the animals behavioural signs in control and test groups. Mortality noted in 2 animals at the dose level of 300mg/kg body weight 3 to 6 hours after the drug administration.

Gross Necropsy:

1. No abnormalities seen in external observation and necropsy examinations on the dose level of 5mg/kg bw.and 50mg/kgbw.
2. No abnormalities seen in external observation and necropsy examinations on the dose level of 50mg/kgbw.
3. In the dose level of 300mg/kg bw, mortality was noted in two animals in three to six hours. Colloid like defecations (3-4times), nasal discharge, clonus with hyper extension, fixed eyeballs were observed. Necropsy showed, frothy discharge from trachea, lung congestion, hyperemia in fundus of the stomach, bulged intestines were observed. Other vital organs were normal.

REPEATED ORAL TOXICITY STUDY

Clinical signs

No abnormal behavioural signs were observed during the study period.

Mortality

The test drug Puttru Pathangam did not cause any mortality in X, 5X and 10X dose levels and were considered as safe dose levels. The X dose was calculated with the human dose and body surface area of rats.

Body weight

Both control and test dose groups exhibited normal body weight throughout the study period. Table. (6).

Food consumption

No difference in food intake of control and test group animals were observed during the period of study. Table. (8)

Water intake

No difference in water intake of control and test group animals were observed during the period of study. Table. (7).

Hematological investigations

The results of hematological investigation conducted at the end of the study, test groups revealed no significant changes in values of different parameters, when compared with control group. The Lymphocyte count was slightly elevated in test groups, but statistically not significant when compared with control group. Table. (9).

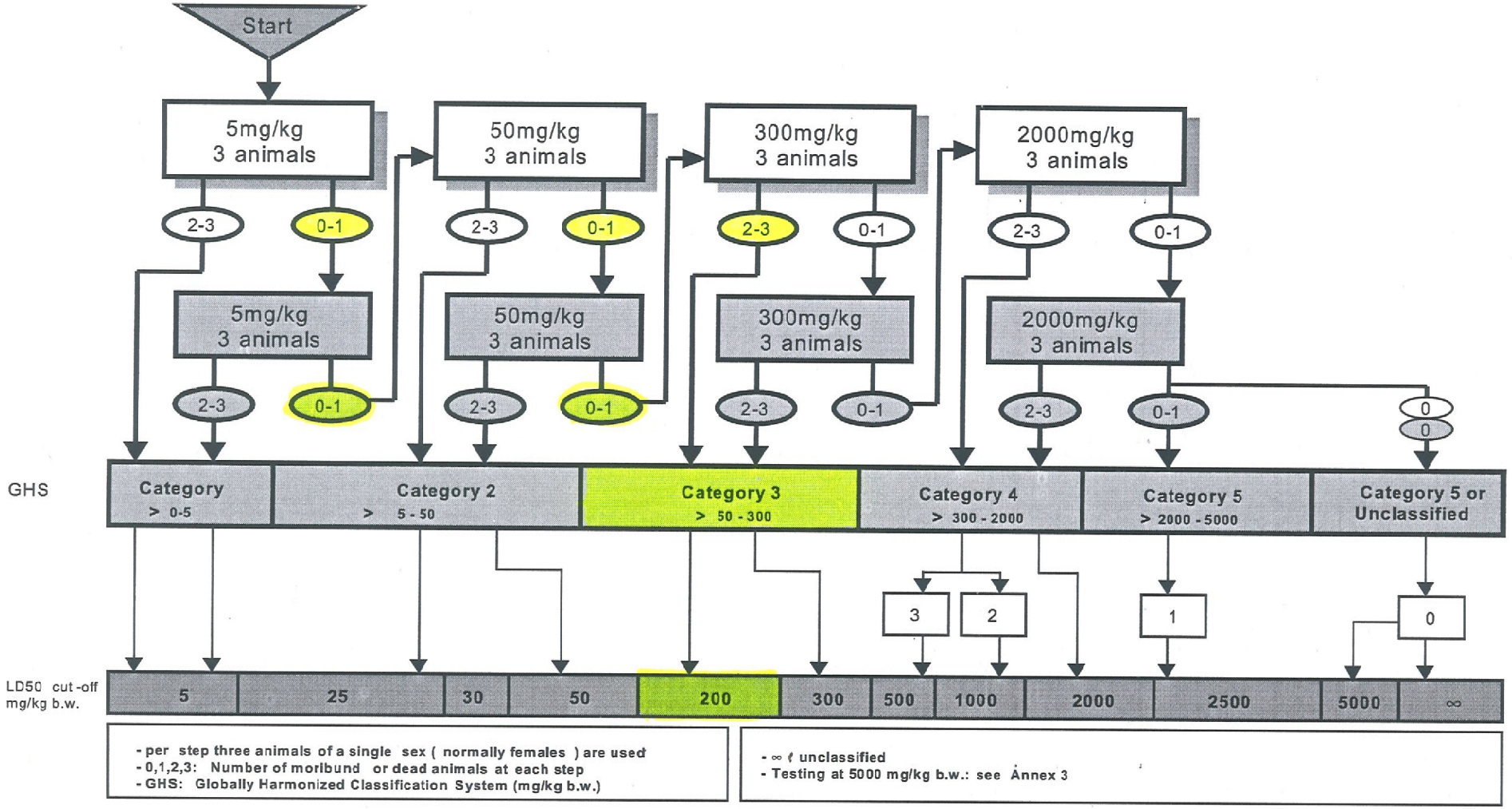
Biochemical investigations

Biochemical investigations were conducted at the end of the study and the results were recorded. In test groups there was no significant elevation in the levels of biochemical parameters, when compared with the control group. And the values obtained were within normal biological limits. Table. (10 - 12)

Histopathology

Gross pathological examination of animals doesn't reveal any abnormalities in control and test groups. The histopathological study of the organs such as heart, lungs, kidney, spleen, stomach and liver was normal in control, X, and 5X groups. In 10X group, lung shows focal lymphoid aggregation. Liver shows portal tract with focal lymphoid infiltrate, radiating cords of hepatocytes, sinusoids were normal. Stomach shows acute and chronic inflammatory cells in lamina propria. Heart shows normal myocardial fibers with patterned coronaries.

ANNEX 2a: TEST PROCEDURE WITH A STARTING DOSE OF 5 MG/KG BODY WEIGHT



HISTOPATHOLOGICAL REPORT

HEART

Plate .a. CONTROL

Sections shows normal myocardial fibers with patterned coronaries.

Plate .b. X group

Section shows normal myocardial fibers with patterned coronaries.

Plate .c. 5X group

Section shows normal myocardial fibers with patterned coronaries.

Plate .d. 10X group

Section of the heart shows normal myocardial fibers with patterned coronaries

HEART

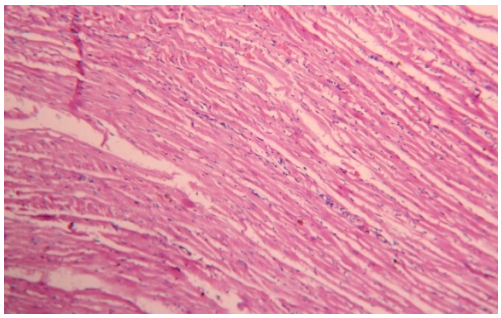


Plate.a.CONTROL

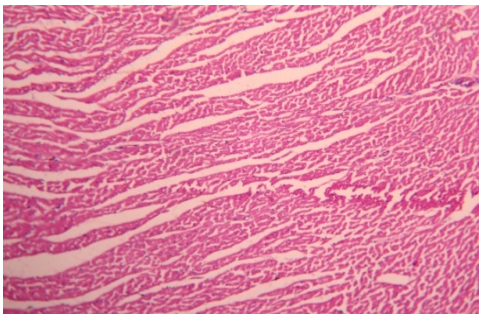


Plate.b.X group

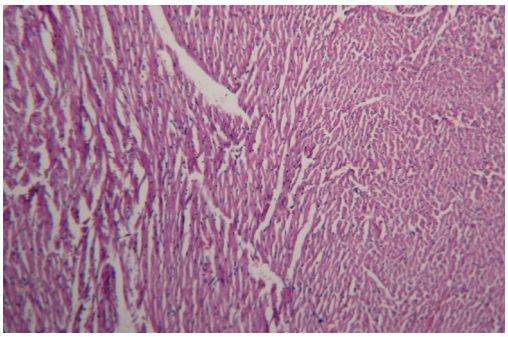


Plate.c. 5X group

Low power

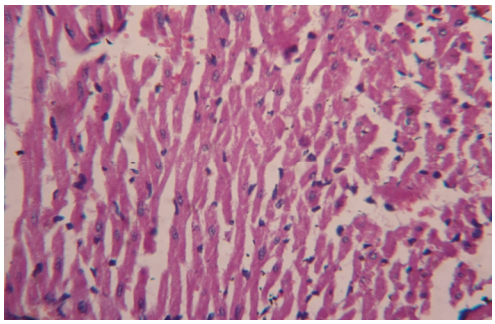


Plate.d.10X group

High power

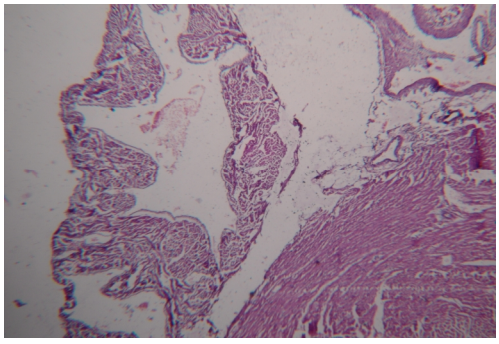


Plate d. 10X group – Scanner

KIDNEY

Plate .a. CONTROL

Specimen shows normal appearing glomeruli, tubules and intestitium.

Plate .b. X group

Specimen shows normal appearing glomeruli, tubules and intestitium..

Plate .c. 5X group

Specimen shows normal appearing glomeruli, tubules and intestitium.

Plate .d. 10X group

Section from the kidney shows normal appearing glomeruli, tubules and intestitium..

KIDNEY

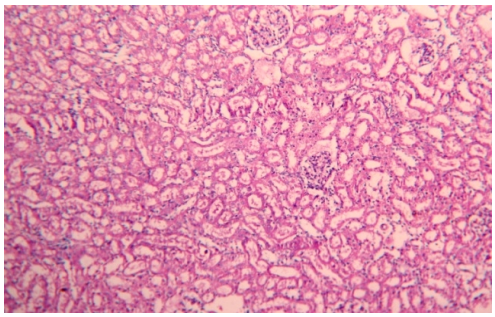


Plate.a. Control

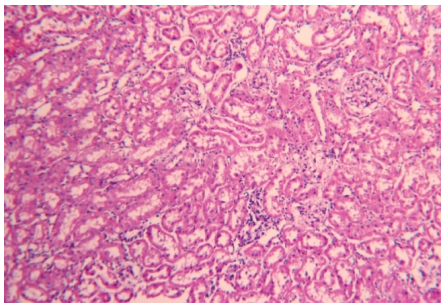


Plate.b. X group

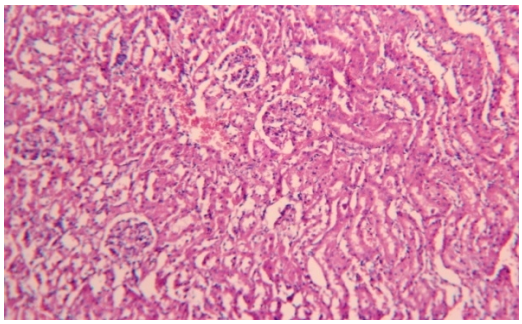


Plate.c. 5X group

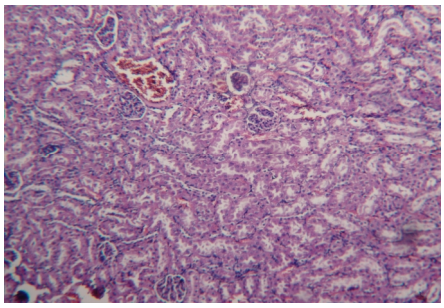


Plate.d. 10X group

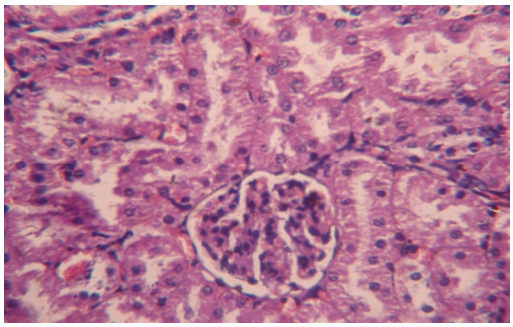


Plate.e.10X group - high power.

LIVER

Plate .a. CONTROL

Section from the liver shows central veins surrounded by radiating cords of hepatocytes separated by sinusoids containing kuffer cells and portal triad shows lymphoid infiltrate.

Plate .b. X group

Section from the liver shows central veins surrounded by radiating cords of hepatocytes separated by sinusoids containing kuffer cells and portal triad shows lymphoid infiltrate.

Plate .c. 5X group

Section from the liver shows central veins surrounded by radiating cords of hepatocytes separated by sinusoids containing kuffer cells and portal triad shows lymphoid infiltrate.

Plate .d. 10X group

Section from the liver shows central veins surrounded by radiating cords of hepatocytes separated by sinusoids containing kuffer cells and portal triad shows lymphoid infiltrate.

LIVER

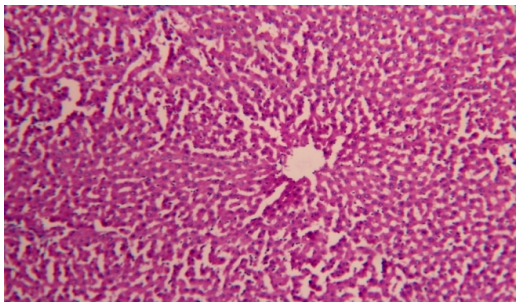


Plate.a. CONTROL

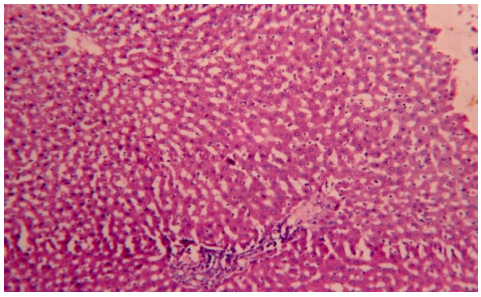


Plate.b. X group

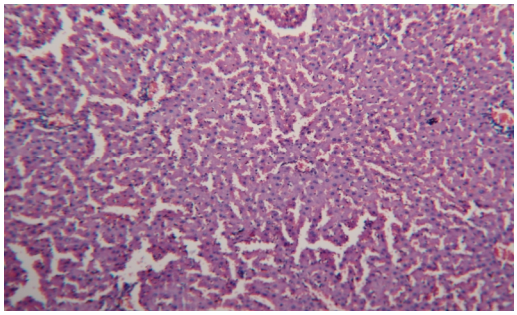


Plate.c.5X group

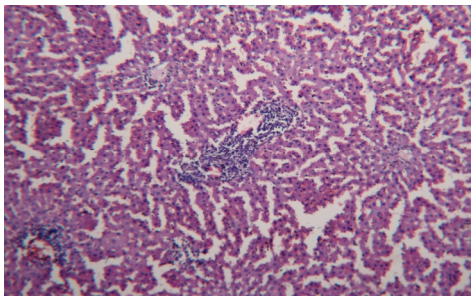


Plate.d.10X group

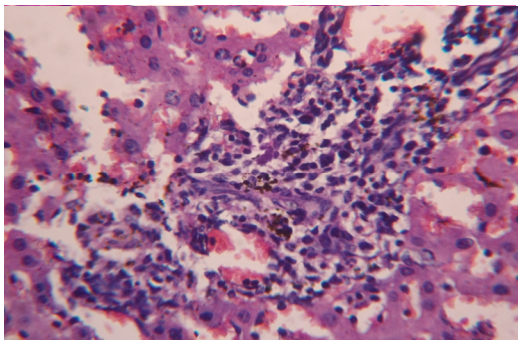


Plate.e.10X group - high power

shows lymphoid infiltrate

LUNG

Plate .a. CONTROL

Section from the lung shows normal appearing bronchioles, alveoli, intestitium and blood vessels.

Plate .b. X group

Section from the lung shows normal appearing bronchioles, alveoli, intestitium and blood vessels.

Plate .c. 5X group

Section from the lung shows normal appearing bronchioles, alveoli and focal lymphoid aggregates.

Plate .d. 10X group

Section of the lung shows normal appearing bronchioles, alveoli and focal lymphoid aggregates.

LUNG

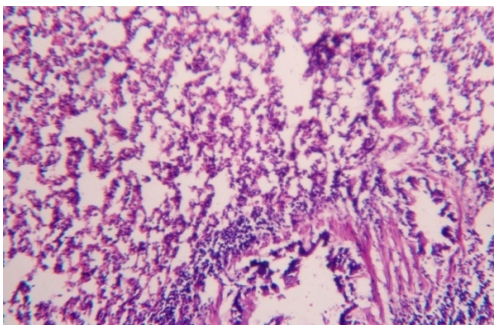


Plate.a. CONTROL

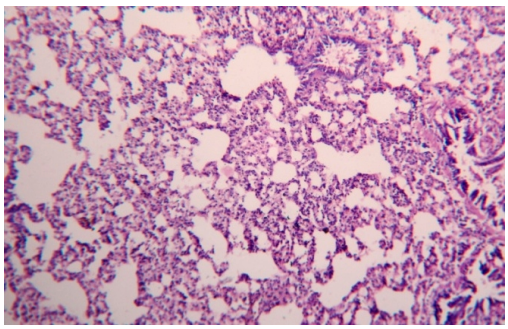


Plate.b. X group

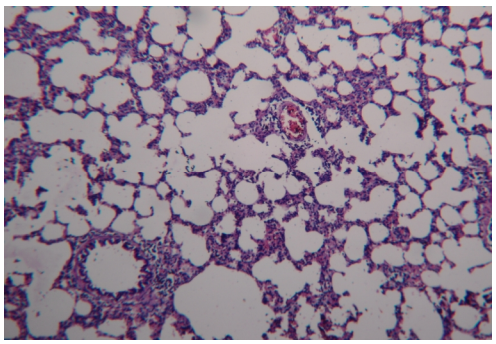


Plate.c.5X group

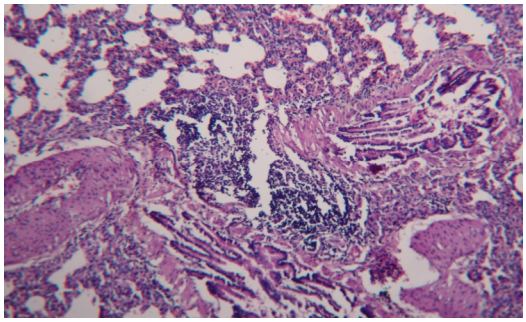


Plate.d. 10X group

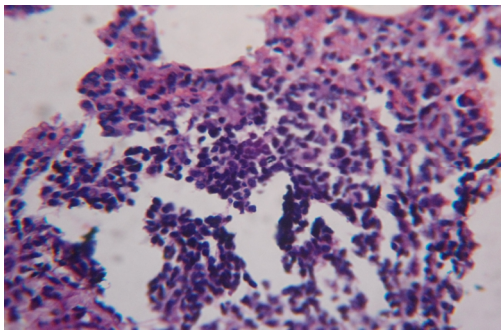


Plate.e. shows lymphoid infiltrate

STOMACH

Plate .d. 10 Xgroup

Section of the stomach shows gastric mucosa lined by tall columnar cells. The mucosa is ulcerated. Lamina propria shows diffused acute and chronic inflammatory cells. Muscularis mucosae and muscularis propria are normal.

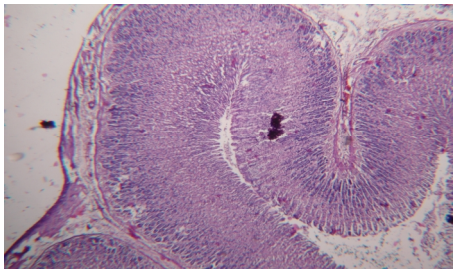


Plate.d. 10 X scanner image

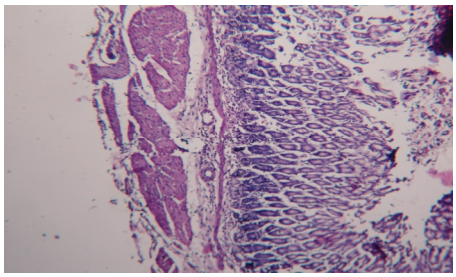


Plate.d. 10 X ulcerated area

SPLEEN

Plate .d. 10X group

Section from the spleen shows white pulp with lymphoid follicles and central arteriole. The red pulp is congested.

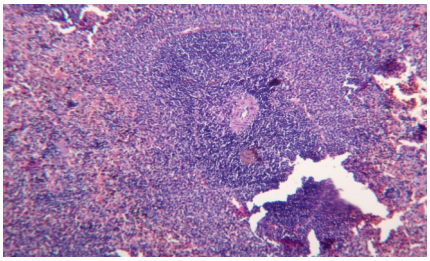


Plate .d. 10X group-low power

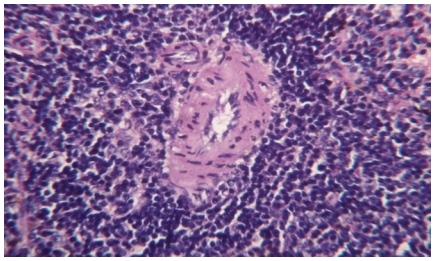


Plate.d. 10X group- central artery.

Table.6. Behavioral signs of Acute toxicity study in Swiss albino mice treated with Puttru Pathangam

No.	Treatment group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	Control	+	–	–	+	+	+	–	–	–	+	–	+	+	+	–	–	–	–	N	A
2.	5mg/kg	+	–	–	+	+	+	+	–	–	+	+	+	+	+	–	–	–	–	N	A
3.	50mg/kg	+	–	–	+	+	+	+	–	–	+	+	+	+	+	–	–	–	–	N	A
4.	300mg/kg	+	–	–	+	–	–	–	–	+	–	+	–	–	–	+	+	+	–	N	+

1. Alertness 2. Aggressiveness 3. Passivity. 4. Grooming 5. Gripping strength 6. Touch responses 7.Restlessness 8. Tremors 9. Convulsion 10. Pain response 11.Defaecation 12. Pinna reflex13.Corneal reflex 14.Pupillary size15. Lacrimation 16. Salivation 17. Urination 18. Writhing 19.Skin colour 20. Mortality

+ Presence of activity, N - Normal,

–Absence of activity A - Absent

Table.7. Body wt (g) of Wister rats in Repeated oral dose toxicity study treated with Puttru Pathangam

DOSE (mg/animal)	DAYS				
	1	7	14	21	28
CONTROL	119.50 ± 5.30	115.60 ± 5.03	118.05 ± 3.44	122.50 ± 4.54	130.15 ± 3.24
X - GROUP	112.33 ± 2.36	114.01 ± 2.66	120.48 ± 3.44	124.60 ± 4.15	132.13 ± 4.56
5X - GROUP	115.45 ± 4.12	118.23 ± 3.44	119.86 ± 3.50	132.77 ± 3.33	134.19 ± 4.53
10 X - GROUP	114.40 ± 3.89	118.22 ± 2.25	124.76 ± 3.19	133.47 ± 5.55	135.13 ± 4.58
P value (p)*	NS	NS	NS	NS	NS

NS- Not Significant, *($p > 0.05$), n = 6 values are mean ± S.D (One way Anova followed by Dunnett's test)

Table.8. Water intake (ml/day) of Wister rats in Repeated oral dose toxicity study treated with Puttru Pathangam

DOSE (mg/animal)	DAYS				
	1	7	14	21	28
CONTROL	35.55 ± 2.54	29.60 ± 2.83	34.05 ± 3.59	34.5 ± 4.57	30.15 ± 3.24
X - GROUP	31.33 ±7.44	31.01 ± 2.66	34.48 ± 4.17	32.61 ± 4.15	37.23 ± 3.44
5X - GROUP	29.47 ± 3.89	40.23 ± 3.36	31.86 ± 3.89	36.77 ± 3.33	38.19 ± 4.53
10 X - GROUP	33.49 ± 4.58	39.22 ± 4.25	36.76 ± 3.09	40.47 ± 5.55	40.13 ± 4.12
P value (p)*	NS	NS	NS	NS	NS

N.S- Not Significant, *(p >0.05), n = 6 values are mean ± S.D (One way Anova followed by Dunnett's test)

Table.9. Food (g/kg) intake of Wister rats in Repeated oral dose toxicity study treated with Puttru Pathangam

DOSE (mg/animal)	DAYS				
	1	7	14	21	30
CONTROL	29.55 ± 3.24	33.60 ± 3.55	39.05 ± 3.25	41.5 ± 2.05	43.15 ± 4.76
X - GROUP	26.33 ±4.15	39.01 ± 2.66	34.48 ± 3.44	38.6 ± 3.89	41.32 ± 4.11
5X GROUP	25.47 ± 4.12	35.23 ± 3.44	38.86 ± 3.89	35.77 ± 2.36	44.19 ± 4.53
10 X GROUP	28.49 ± 3.33	31.22 ± 4.25	35.76 ± 3.09	33.47 ± 5.58	41.13 ± 4.58
P value (p)*	NS	NS	NS	NS	NS

N.S- Not Significant,* (p >0.05),n = 6 values are mean ± S.D (One way Anova followed by Dunnett's test)

Table.10. Haematological parameters of Wister rats in Repeated oral dose toxicity study treated with Puttru Pathangam

Category	Haemoglobin (g/dl)	Total -WBC (cells/cu.mm)	Diff.Count Neutrophil (%)	Diff.Count Lymphocyte (%)	Total - RBC (cells/cu.mm)
CONTROL	13.4 ± 0.6	7056 ± 907	23.3 ± 15.3	55.4 ± 24.1	7.2 ± 1.2
X	15.5 ± 2.2	8250 ± 1203	21.1 ± 9.3	68.5 ± 9.2	7.8 ± 1.2
5X	16.4 ± 3.5	7850 ± 805	24.7 ± 10.1	70.6± 8.3	8.1 ± 1.0
10X	15.0 ± 2.5	9765 ± 1076	24.5 ± 8.3	77.3 ± 10.3	7.9 ± 0.3
P value (p)*	NS	NS	NS	NS, ** 5X vs. X -S	NS

NS- Not Significant,* ($p > 0.05$), ** S – Significant. ($p < 0.05$). n = 6 values are: mean ± S.D
(One way Anova followed by Dunnett's test)

Table.11. Biochemical Parameters of Wister rats in Repeated oral dose toxicity study treated with Puttru Pathangam

BIOCHEMICAL PARAMETERS	CONTROL	X GROUP	5X GROUP	10X GROUP	P Value (p)*
GLUCOSE (R) (mg/dl)	89.2 ± 28	95.2±15	116 ± 37	93 ± 25	N.S
T.CHOLOSTEROL(mg/dl)	86.1 ± 7.4	70. ±10	87 ± 5.2	91 ± 4.3	N.S
HDL(mg/dl)	20.2 ± 4.3	23 ± 5.3	22 ± 7.6	23 ± 2.1	N.S
LDL(mg/dl)	23.1 ± 5.7	22 ± 8.3	20 ± 6.5	22 ± 6.3	N.S
VLDL(mg/dl)	27.5 ± 4.5	24 ± 4.4	26 ± 6.4	26 ± 5.3	N.S
TRIGLY(mg/dl)	132.4 ± 20	127 ± 17	124 ± 28	129 ±17	N.S

NS- Not Significant,* ($p > 0.05$), n = 6 values are mean ± S.D (One way Anova followed by Dunnett's test)

Table.12. Renal function test of Wister rats in Repeated oral dose toxicity study treated with Puttru Pathangam

PARAMETERS	CONTROL	X - GROUP	5X - GROUP	10X - GROUP	P Value (p)*
UREA (mg/dl)	10 ± 5.5	16 ± 3.5	18.3±7.5	10.4 ± 6	N.S
CREATININE (mg/dl)	0.76 ± 0.23	0.59 ± 0.15	0.76 ± 0.19	0.62 ± 0.12	N.S
URIC ACID(mg/dl)	2.71 ± 0.23	3.67 ± 0.36	2.38 ± 0.55	2.73 ± 0.47	N.S
CALCIUM(mg/dl)	8.5 ± 0.8	8.9 ± 1.8	7.7 ± 0.6	9.1 ± 2.3	N.S
POTASSIUM (mg/dl)	2.6 ± 0.4	3.7 ± 0.5	2.6 ± 0.5	3.1 ± 0.6	N.S

NS- Not Significant,* ($p > 0.05$) , n = 6 values are mean ± S.D (One way Anova followed by Dunnett's test)

Table.13. Liver Function Test of Wister rats in Repeated oral dose toxicity study treated with Puttru Pathangam

PARAMETERS	CONTROL	X - GROUP	5X - GROUP	10X - GROUP	P Value (p)*
T.BILIRUBIN(mg/dl)	0.4 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	N.S
D.BILIRUBIN(mg/dl)	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.3	0.3 ± 0.1	N.S
I. BILIRUBIN(mg/dl)	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	N.S
SGOT(U/dl)	67 ± 24	64 ± 23	58 ± 16	57 ± 16	N.S
SGPT(U/dl)	67 ± 11	76 ± 25	66 ± 23	72 ± 22	N.S
ALP(U/dl)	132 ± 6	142 ± 15	138 ± 18	134 ± 20	N.S
T.PROTEIN(mg/dl)	6.5 ± 0.9	6.7 ± 0.3	5.7 ± 0.5	6.9 ± 0.3	N.S
ALBUMIN(mg/dl)	3.1 ± 0.6	4.1 ± 0.5	3.2 ± 0.3	3.6 ± 0.4	N.S
GLOBULIN(mg/dl)	3.2 ± 0.7	2.7 ± 0.2	2.5 ± 0.7	3.6 ± 0.3	N.S

NS- Not Significant,* ($p > 0.05$), n = 6 values are mean ± S.D (One way Anova followed by Dunnett's test)

Chart.1. Mean arithmetic values of Control and test group animals

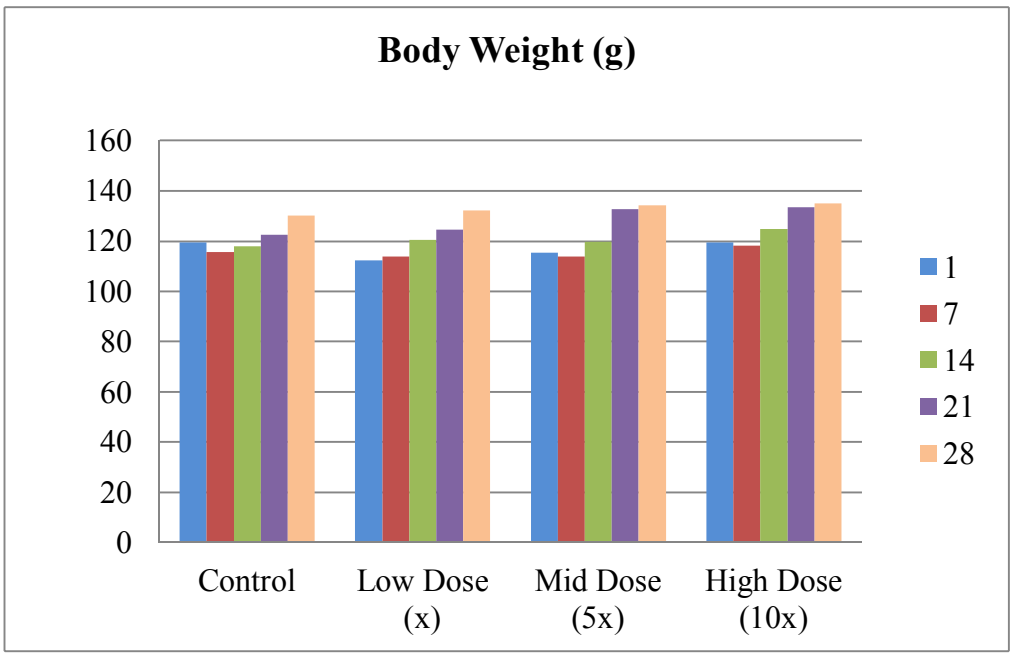


Chart.2. Mean arithmetic values of Control and test group animals

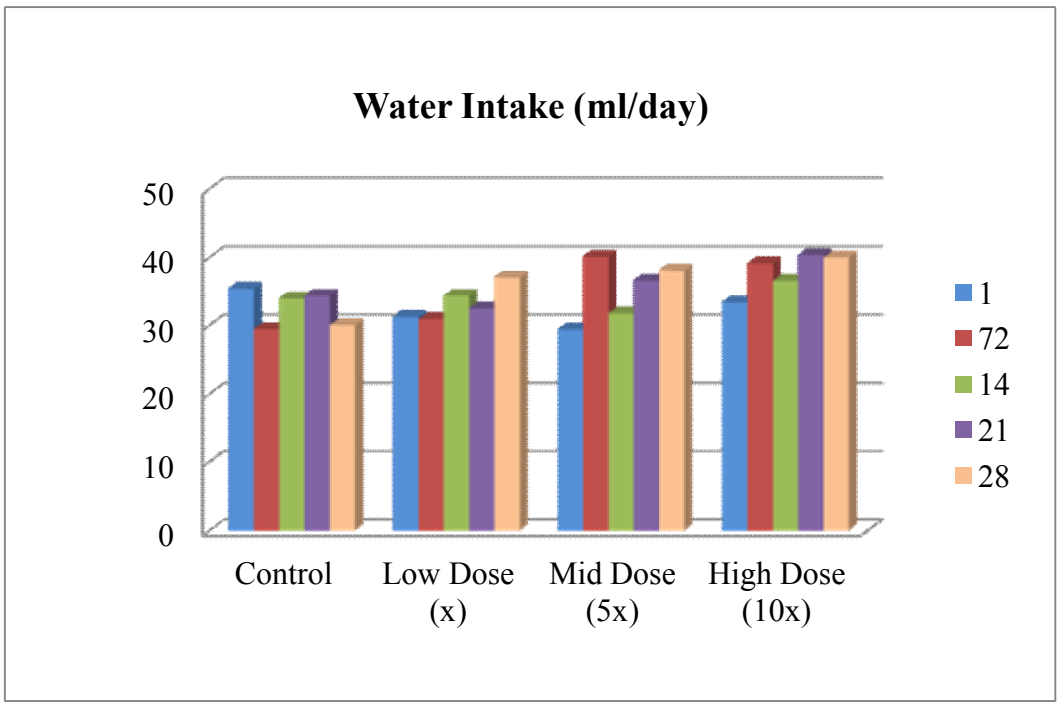


Chart.3. Mean arithmetic values of Control and test group animals

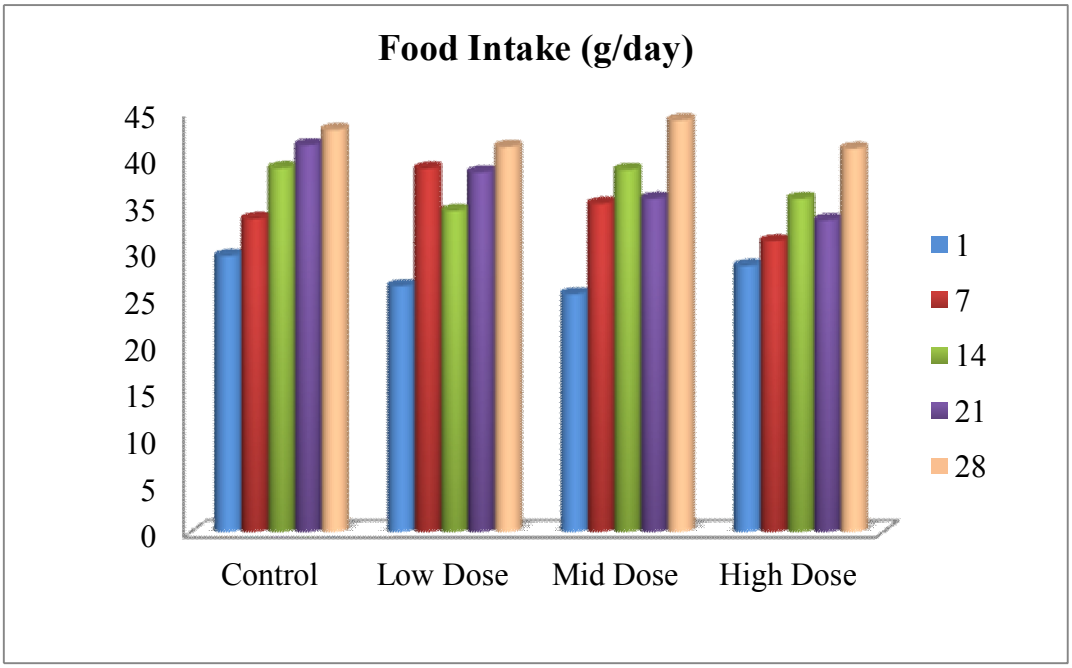


Chart.4. Mean arithmetic values of Control and test group animals

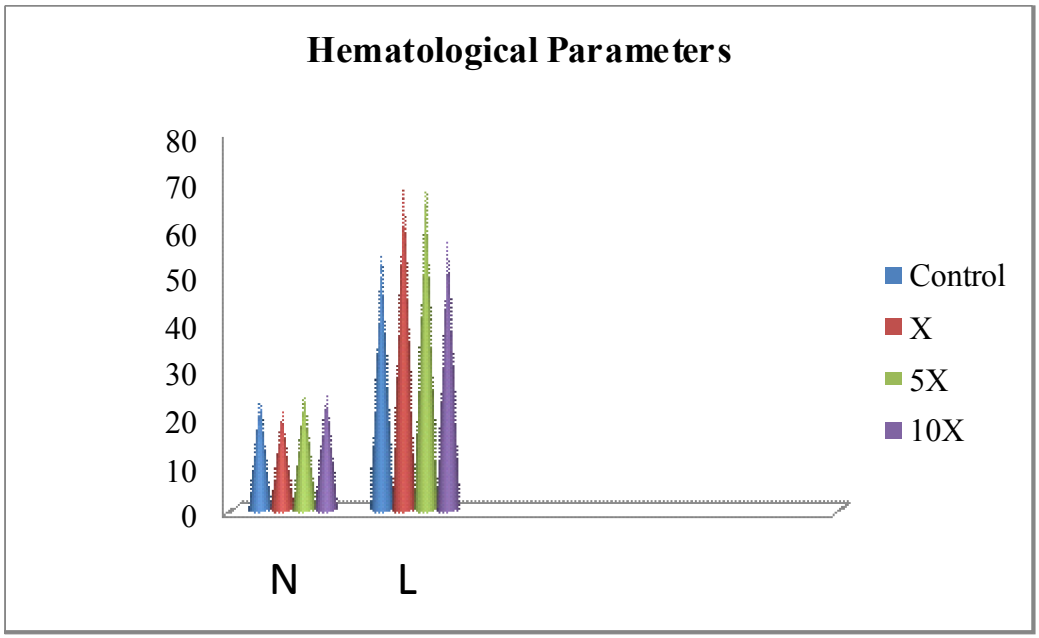
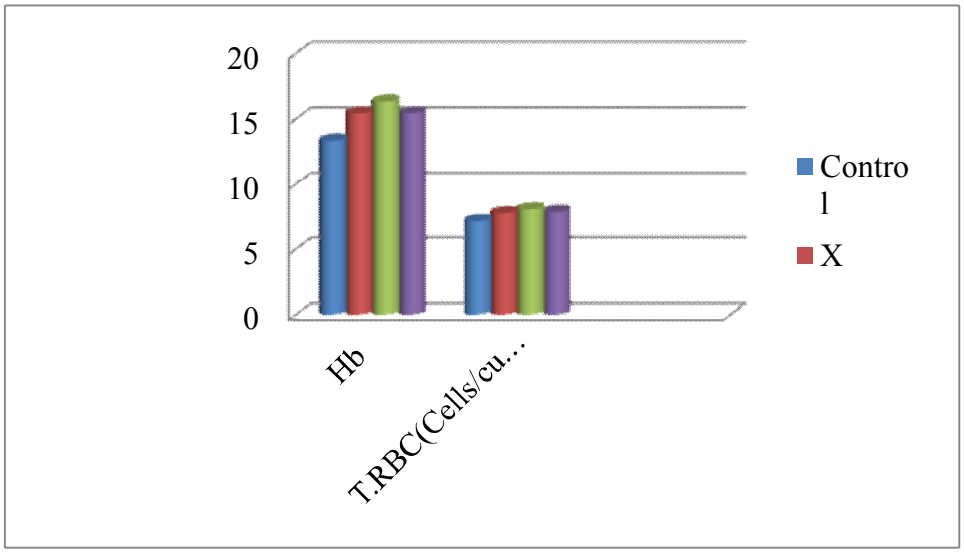


Chart.5. Mean arithmetic values of Control and test group animals



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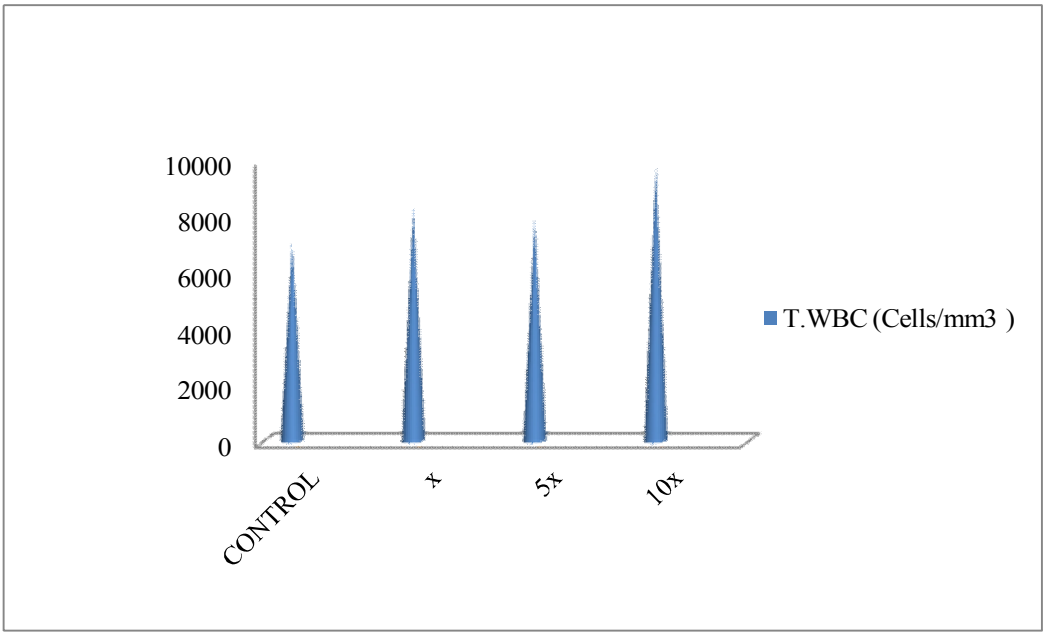


Chart.7. Mean arithmetic values of Control and test group animals

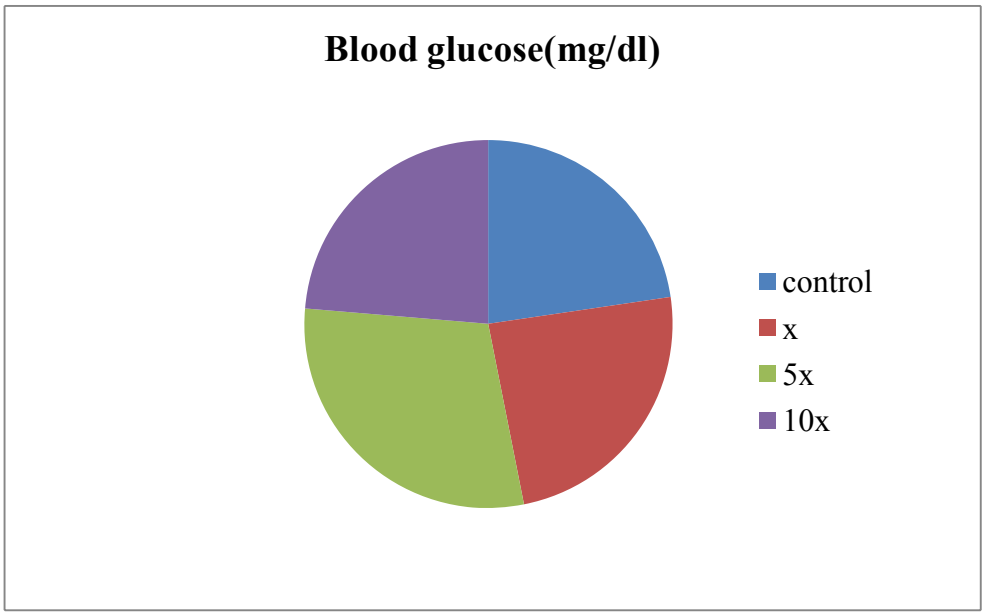
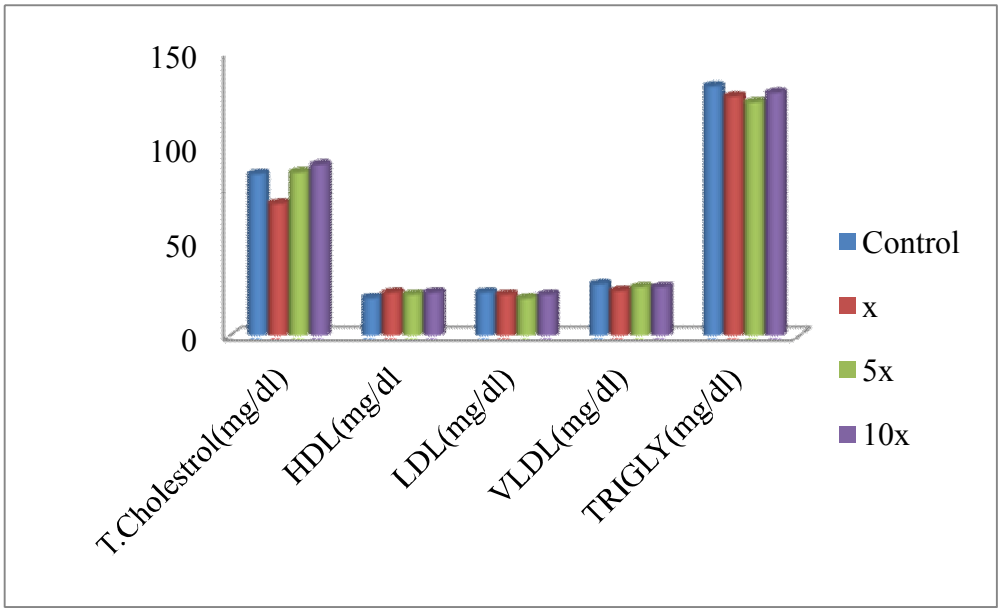


Chart.8. Mean arithmetic values of Control and test group animals



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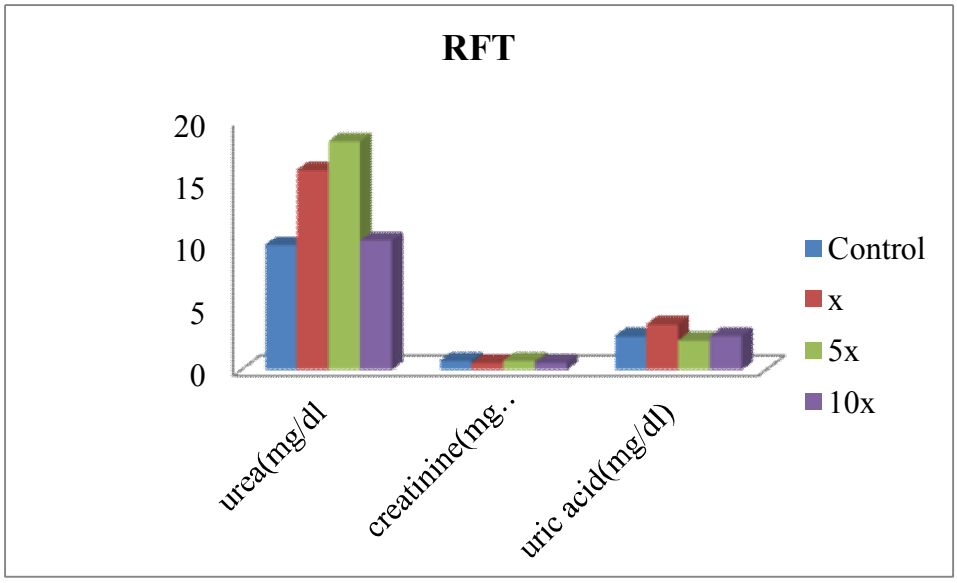

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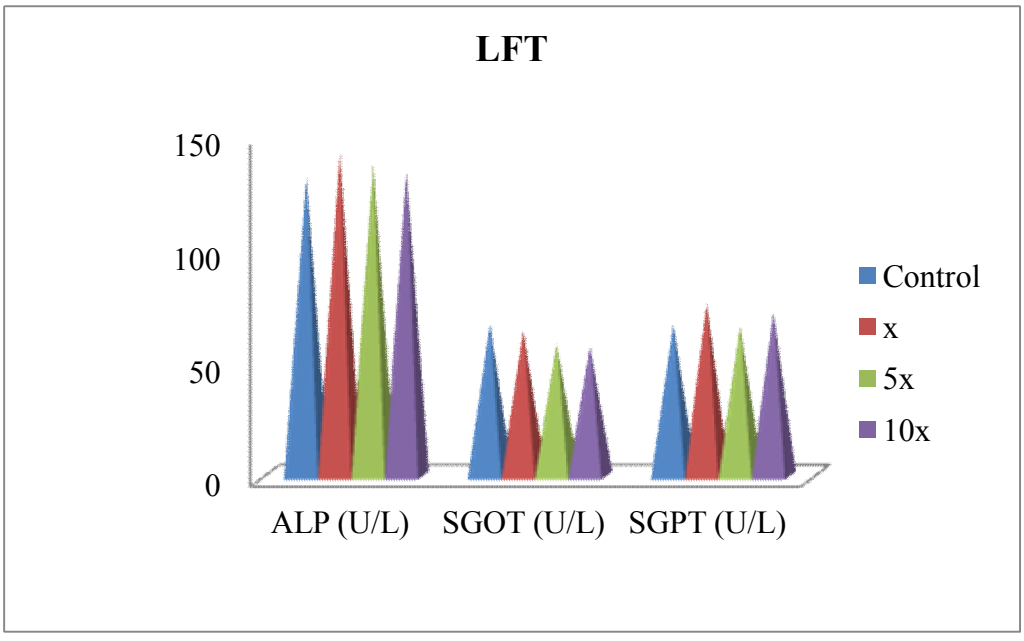

In this study Puttru Pathangam was subjected to qualitative, quantitative and toxicity studies. Qualitative analysis includes physico chemical properties of Puttru Pathangam. Quantitative analysis includes ICP-OES, XRF, FTIR and SEM. And then finally both Acute and Repeated oral dose toxicity study were carried out in rodents as per OECD guidelines.

Qualitative Analysis of the Puttru Pathangam showed the presence of phosphate, Iron, chloride and unsaturated compound.

In the **Physico Chemical Analysis,** the pH of Puttru Pathangam was 3.2 -3.5. It denotes acidity. Thus, on oral intake it may be easily absorbed from the gastrointestinal tract. The loss on drying at 105ºC was only 13.34% w/w; hence the drug will not lose much of its volume on exposure to atmospheric air at room temperature. Solubility of Puttru Pathangam was found to be increased compared to the purified and unpurified ingredients of it. Hence there is possibility of increased absorption.

In **ICP-OES** study, heavy metals like As, Pb, Cd were found below detection limit in Puttru Pathangam. Mercury, calcium, iron, potassium, phosphorous, sodium were present. (Table.4)

In **XRF** analysis (Table.5), the heavy elements in Puttru Pathangam were within normal limits when compared to ICP OES.

FTIR analysis of Puttru Pathangam indicates the addition of primary and secondary amines, aromatics, alkyl halides and alkynes. This indicates the drug attained to fulfill its therapeutic value.(Table.6)

HR SEM analysis of Puttru Pathangam reveals that the particle size is 0.25-0.5μ(micron). The particles were homogenously distributed in the Pathangam. And smooth surface of the particles enable easy absorption in the gastro intestinal tract. Hence the drug will have increased bioavailability.(Fig.4.)

Mercury was detected in level in the finally prepared medicine Puttru Pathangam as (3.842). But the arsenic as an ingredient, it was found below the detection limit. This

indicates that all heavy metals in Puttru Pathangam were within normal range and near to permissible limit. So that Puttru Pathangam is safe for human consumption. (Table.4.)

In **Acute oral dose toxicity study** period there were no abnormal signs developed in 5mg 50mg treated animals. No mortality and reduction in body weight of animals were observed in 5mg and 50mg groups. In the group of 300mg/kg, very high dose when compare with Human therapeutic dose (65mg), mortality was observed in two animals. So the LD_{50} of Puttru Pathangam is 200mg/kg body weight as per OECD guidelines. (Table.7.)

At the end of **Repeated oral dose toxicity study** animals were sacrificed and blood samples were collected and investigated. The organs were collected and sent for histopathology study. All the reports were statistically calculated. There were no significant changes in hematological parameters, biochemical investigations, body weight, food and water intake. There was a marked increase in lymphocyte count in test groups, but compared to control group, it is not statistically significant. (Table.8-13).

Gross pathological examination of animals doesn't reveal any abnormalities in control and test groups.

The **histopathological** study of the organs such as heart, lungs, kidney, spleen, stomach and liver was normal in control, X, and 5X groups. In 10X group, lung shows focal lymphoid aggregation. Liver shows portal tract with focal lymphoid infiltrate, radiating cords of hepatocytes, sinusoids were normal. Stomach shows acute and chronic inflammatory cells in lamina propria. Heart shows normal myocardial fibers with patterned coronaries.

Siddha system is the ancient traditional system followed in South India mainly in Tamilnadu. In Siddha system of medicine Plants, minerals and Metals play an important role. Mercurial compound has also been practised in Siddha system from time immemorial. Rasam, Gandhagam, Lingam, Thaalagam, Manosilai, Vellai paadanam, Pooram, Gaantham and Saathikai.Puttru Pathangam is one of the notable medicine in the treatment of cancer. The name itself tells. Puttru Pathangam taken from the literature **Anuboga Vaithiya Navaneetham, part-10,** is also used in the treatment of various types of cancer as in cheek, breast, mouth cancer, sexual organs, cancer in legs, vellai kuttam (vitiligo) and kandamaalai (cervical adenitis).

The raw drug was procured from country drug shop from Kanyakumari and authenticated at Siddha Central Research Institute and the plant drugs authenticated in Department of Medicinal Botany in NIS. The drugs were purified and the medicine was prepared as mentioned in the Siddha literature at Gunapaadam laboratory in NIS.

Chemical analysis of the drug Puttru Pathangam confirmed the presence of phosphate, Iron, chloride and unsaturated compound.

In physico - chemical analysis of Puttru Pathangam the pH was found to be 3.2 - 3.5 and the loss on drying at 105ºC was 13.34% w/w.

In ICP-OES study, heavy metals like As, Pb, Cd were found below detection limit in Puttru Pathangam. Mercury, calcium, iron, potassium, phosphorous, sodium were present. Mercury was detected in minimal level in the finally prepared medicine Puttru Pathangam as (3.842).

In **XRF** analysis the heavy elements in Puttru Pathangam were found within normal limits when compared to ICP OES.

By the HR SEM analysis, the particle size of the Puttru Pathangam was analyzed as 0.25-0.5 µ (micron).

The toxicological evaluations were conducted at Animal house of NIS, as per OECD guidelines for safety evaluation of Puttru Pathangam.

In Acute oral dose toxicity study period there were no abnormal signs developed in 5mg 50mg treated animals. No mortality and reduction in body weight of animals were observed in 5mg and 50mg groups. In the group of 300mg/kg, very high dose when compare with Human therapeutic dose (65mg), mortality was observed in two animals. So the LD_{50} of Puttru Pathangam is 200mg/kg body weight as per OECD guidelines.

At the end of Repeated oral dose toxicity study animals were sacrificed by excessive anesthesia and blood samples were collected and investigated. The organs were collected and sent for histopathology study. All the reports were statistically calculated. There were no significant changes in hematological parameters, biochemical investigations, body weight, food and water intake.(Table 7-13) There was a marked increase in lymphocyte count in the test groups, but compared to control group, it is not statistically significant. (Table.10)

As the result of this study, it has been concluded that

Though the white arsenic is an ingredient, it was found to below the detection level in the prepared medicine analyzed by sophisticated instrument. It reveals the potential background of Siddhars knowledge in the field of metals, minerals and herbal usage in medicine preparation. Mainly the mercurial content of the prepared medicine Puttru Pathangam showed is less as an ingredient (3.842).

The acute and repeated oral dose toxicity of Puttru Pathangam is found to be less toxic and the therapeutic dose level mentioned in the literature is a safer dose for human consumption.

Hence further studies on Puttru Pathangam(Indicated mainly for cancer) are to be conducted for its scientific validation and global acceptance.

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IAEC PROTOCOL NO. 1248/ac/09/CPCSEA/4-38/2011

20/12/2011

CERTIFICATE

This is certify that the project title. A toxicity study on "PUTTRU PATHANGAM" **has been approved by the IAEC.**

Dr. K. MANICKAVASAKAM
Name of Chairman/Member Secretary IAEC:

Dr. B. JAYACHANDRAN DARE
Name of CPCSEA nominee:

Signature with date

Chairman/Member Secretary of IAEC:

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

CERTIFICATE OF BOTANICAL AUTHENTICITY

Certified that the following plant drugs used in the formulation **"Puttru Pathangam"** (Internal) for **toxicity studies** taken up for Post Graduation Dissertation by **Dr.S.Shanmugadevi**, M.D.(S), III year, Department of Nanjunoolum Maruthuva Neethinoolum, 2012-13, are identified and authenticated through Visual inspection / Experience, Education & Training / Organoleptic characters / Morphology / Taxonomical / Microscopical methods.

Myristica fragrans Houtt. (Myristicaceae), Seed
Acalypha indica Linn. (Euphorbiaceae), Whole plant
Alternanthera sessilis (Linn.) R. Br. (Amaranthaceae), Whole plant
Leucas aspera Spreng. (Lamiaceae), Whole plant
Citrus limon (Linn.) Burm. f. (Rutaceae), Fruit
Piper nigrum Linn. (Piperaceae), Fruit
Piper betle Linn. (Piperaceae), Leaf
Amaranthus tricolor Linn. (Amaranthaceae), Whole plant

MEDICINAL BOTANY CHENNAI 600 047 National Institute of Siddha

Certificate No: NIS/MB/65/2012

Date: 28-8-12

Authorized Signatory
Dr. D. ARAVIND, M.D.(s),M.Sc.,
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Department of Medicinal Botany
National Institute of Siddha
Chennai - 600 047, INDIA

சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம், அரும்பாக்கம், சென்னை - 600 106
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E-mail: crsiddha@gmail.com
URL: www.crsiddha.tn.nic.in

08.10.2012

CERTIFICATE

Certified that the minerals submitted for identification by Dr. S. Shanmuga Devi, III year M.D., Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Tambaram Sanatorium, Chennai-47 are identified as Kaantham – Ferric oxide, Manosilai – Arsenic disulphide, Aridharam - Arsenic trisulphide, Vellai Paadaanam – Arsenic trioxide, Rasam – Mercury, Poorum – Mercurous chloride, Lingam – Mercuric sulphide, Ganthagam – Sulphur and Common salt – Sodium chloride.

(R.Shakila)
Research Officer (Chemistry)

(S. Jega Jothi Pandian)
Asst. Director- In charge

The Tamil Nadu Dr. M.G.R. Medical University

69, Anna Salai, Guindy, Chennai-600 032

This Certificate is awarded to ~~Mr/Ms~~/Dr.....S. SHANMUGADEVI.....

for participating as a ~~Resource Person~~ / Delegate in the VII Workshop

on **"Research Methodology & Biostatistics"**

for AYUSH Post-Graduates & Researchers

organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University

from 6th Feb. 2012 to 10th Feb. 2012.

DR. MAYILVAHANAN NATARAJAN
M.S.Orth. M.Ch.Orth. (L'pool) Ph.D. (Orth. Onco.) F.R.C.S. (Eng) D.Sc.
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INDIAN INSTITUTE OF TECHNOLOGY, MADRAS
Chennai - 600 036. INDIA

CERTIFICATE

Certified that herbo-mineral drug **PUTTRU PATHANGAM** formulated by **Dr.S.Shanmugadevi** III Year M.D(S) Department of Nanjunool ,National Institute of Siddha , Tambaram Sanatorium was analysed (quantitative) by ICP-OES, FT-IR, HR-SEM and Physico chemical Analysis Methods at SAIF, IITM, Chennai-36, during December 2012.

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